***Pak. J. Bot.*, 44(1): 281-288, 2012.**

GENETIC EVOLUTION AND UTILIZATION OF WHEAT GERMPLASM RESOURCES IN HUANGHUAI WINTER WHEAT REGION OF CHINA

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Abstract

To determine the genetic variation of wheat germplasm resources and improve their use in wheat breeding, 215 wheat cultivars and advanced lines from the Huanghuai Wheat Region of China were used to identify 14 agronomic traits and 7 quality traits, as well as the evolution and utilization of high molecular weight glutenin subunits (HMW-GS) and low molecular weight-glutenin subunits (LMW-GS). From landrace cultivars to current cultivars there had been significant increases in grain numbers spike^{-1}, grain weight spike^{-1}, 1000-kernel weight, grain weight plant^{-1}, spikelet number spike^{-1}, sterile spikelet numbers spike^{-1}, flag leaf width, and flag leaf area. There had been significant decreases in spike number plant^{-1}, plant height, the first internode length, flag leaf length, kernel protein content and wet gluten content. Based on Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) results, a novel HMW-GS combination 20/8 was identified in 1B chromosome of Chinese landrace cultivar Heputou. Subunits 22, 20/8, 2.2+12, and GluB3a were only found in cultivars before the 1960s, and subunits 6+8, 13+16, 3+12, and 4+12 were only found in the cultivars after the 1980s. The average diversity index of 21 traits and allele variance of HMW-GS showed a decreasing-increasing-decreasing tendency. HMW-GS and LMW-GS combination-type cultivars showed an increasing-decreasing tendency. Before the 1980s, most parental strains were from foreign cultivars and landrace cultivars, while after the 1980s, most parental strains were from released cultivars and germplasm created by distant hybridization. This study provided useful information for improvement of wheat breeding in Huanghuai winter wheat region.

Introduction

Germplasm diversity is usually estimated from pedigree, agronomic, and morphological characteristics, and has played an important role in traditional breeding (Nasir *et al*., 2011). The wider the genetic diversity, the faster will be the improvement of cultivars. Narrow germplasm diversity is an obstacle to plant breeding and reduces the ability of plants to resist unsuitable environments. During varietal improvement, directional selection and the creation of novel germplasm can affect genetic diversity. Concern has been focused on the genetic diversity to widen the genetic resource. Roussel *et al.,* (2005) studied 480 bread wheat cultivars originating from 15 European geographical areas (released between 1840 and 2000) with a set of 39 microsatellite markers. They showed that the total number of alleles was quite stable until the 1960s, after which it decreased. The more recent the cultivars, the more similar they were to each other. Liu *et al.,* (2000) examined the genetic diversity of local and breeding cultivars of wheat in China and concluded that there was an abundant genetic diversity among cultivars for characteristics relating to spikes, diseases, and qualitative characters. The diversity of breeding cultivars was slightly less than that of local cultivars in general, but the direction of the genetic variation was obvious different (Liu *et al.,* 2000). According to Landjeva *et al.,* (2006), the genetic diversity of Bulgarian winter wheat was high from 1925–2003, with a value of 0.64 for bread cultivars before 1960 and 0.71 in the 1990s, revealing no declining trends in diversity due to breeding activity. Christiansen *et al.,* (2002) evaluated 75 Nordic spring wheat cultivars released during the 20th century with a total of 47 microsatellite primer pairs and showed that genetic diversity in selected wheat cultivars was significantly enhanced by wheat breeding in the first quarter of the 20th century and then fluctuated from a decrease during the second quarter to a later increase. On the basis of reports by Roussel *et al.,* (2005) and Liu *et al.,* (2000), it could be concluded that wheat diversity gradually decreased with the development of modern wheat breading, however, this is not consistent with the report of Landjeva *et al.,* (2006). Even though there have been conflicting reports on wheat genetic diversity, it is clear that breeding activity had a significant effect on the diversity of wheat germplasm.

In China, the Huanghuai wheat region, covering all of Henan and parts of Hebei, Shanxi, Shaanxi, Gansu, Anhui, Jiangsu, and Shandong, is the most important wheat production zone, with 45% of the total harvesting area and 48% of total wheat yield. Before the 1970s, wheat breeding mainly relied on crosses between selected Chinese landraces and elite introductions from the United States, Italy, and other countries. Since the 1970s, wheat breeding has been changed by the substitution of released cultivars for landrace cultivars as parents, which caused a sharp improvement of wheat production and quality. Accompanying the progress in breeding, more attention was paid to the characteristics of the germplasm and its genetic basis. In Sichuan, genetic diversity of wheat germplasm has tended to decrease since the 1970s (Zhang *et al.,* 2002), while in Henan and Shandong, the genetic diversity index of wheat germplasm gradually decreased before the 1990s, increased in the 1990s, and decreased after the 1990s (Wang *et al.,* 2006 and Zhan *et al.,* 2006). Given the very limited information available on the variation of the main agricultural traits, the abundant variation of the Glu-1 and Glu-3 loci and the fine subunit utilization of germplasm resources from the Huanghuai wheat area were identified and analyzed in this study. We hope to characterize the genetic variation of Huanghuai wheat germplasm resources and to provide useful information for Chinese wheat breeding.

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Materials and Methods

Plant materials: A total of 215 wheat cultivars and advanced wheat lines were divided into four periods (before 1961, 1961-1980, 1981-2000, and after 2000, based on the time of introducing for the foreign cultivars were introduced and the time of releasing for the Chinese cultivars) and analyzed for 14 agronomic traits and seven quality traits, as well as the genetic evolution and utilization of HMW-GS and LMW-GS (high and low molecular weight glutenin subunits). Of the surveyed cultivars, 29 (14 foreign cultivars, 6 landrace cultivars and 9 released cultivars), 33 (2 foreign cultivars and 31 released cultivars), 92, and 61 accessions belong to the first (I), second (II), third (III), and fourth (IV) periods, respectively. These accessions were representative of the landmark landraces, historical cultivars and current cultivars in the Huanghuai Winter Wheat Region of China from the 1930s to 2000s, and were collected from the seven provinces of Henan, Shandong, Hebei, Shaanxi, Shanxi, Anhui, and Gansu. The pedigree, source, and introduced or released years of some representative cultivars are shown in Table 1.

Table 1. Pedigrees, sources, released or introduced years, HMW and LMW subunits of representative cultivars.

Variety	Crosses	Source	Breeding or introduce date	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3
Quality	[White Naples/(Fife/White Naples)]	Australia	1923	N	7+9	2+12	f	a
Jiangdongmen	Landrace	Jiangsu	1925	N	7+8	2+12	a	f
Ardito	(Wilhelmina/Rieti)/Akagomughi	Italy	1929	N	22	2+12	a	d
Yangda1817	Landrace	Hebei	1930	N	7+8	2+12	c	f
Villa Glori	(Rieti/Wilhelmina)/Akagomughi	Italy	1930	1	7+8	2+12	c	d
Mentana	(Rieti/Wilhelmina)/Akagomughi	Italy	1932	N	7+8	2+12	b	d
Mazhamai	Landrace	Shaanxi	1940	N	7+8	2+12	a	b
Ceres	Cheyenne/Early Blackhull	America	1946	2*	7+8	5+10	a	h
Bima4	Mazhamai/Quality	Shaanxi	1947	N	7+8	2+12	c	g
Xinong 6028	Xinong 60/Vill Glori	Shaanxi	1948	1	7+8	2+12	c	d
Norin 10	(Daruma/Fultz)/Turkey Red	Japan	1955	1	7+8	2+12	a	d
ПреПгорНая 2	no	Soviet	1955	1	7+9	5+10	a	j
Abbondanza	Autonomia/Fontarionco	Italy	1956	1	7+8	2+12	d	f
Funo	Duecentodieci/damiano	Italy	1956	1	14+15	2+12	b	g
Orofen	[(Niwthatch/Marroqui588)/ (Kenya C9908/Mentana)]/Frontana	Chile	1959	N	7+8	2+12	a	j
Xuzhou 14	Early Premium/Mentana	Jiangsu	1962	2*	7+8	2+12	b	d
Zhengzhou 24	Bima 4/Eearly P1emium	Henan	1962	2*	7+8	5+10	d	d
Nongda 311	Triumph/Yanda 1817	Hebei	1963	2*	7+9	2+12	c	j
Youbaomai	Youzimai/Shidaobsodasanbaibao	Shandong	1964	1	7+9	5+10	c	d
Fengchan 3	Danish 1/Xinong 6028	Shaanxi	1966	N	7+8	2+12	b	h
Sumai 3	Funo/Taiwanxiaomai	Jiangsu	1968	N	7+8	2+12	d	d
Xinyang 12	Abbondanza/Neixiang 5	Henan	1970	1	14+15	5+10	a	d
Aifeng 3	(Xiannong 39/58(18)2)/Fengchan 3	Shaanxi	1971	1	7+9	2+12	d	d
Lovrin 10	(Abbondanza/T1iumph)/Bezostaja 1	Romania	1972	N	7+9	2+12	a	j
Xiaoyan 4	Fengchan 1/Xiaoyan 96	Shaanxi	1972	1	7+8	5+10	d	d
Qingfeng 1	(Xifeng3/Beijing9)/Ganmai4	Gansu	1973	2*	7+8	5+10	a	d
Bainong 3217	(Xinong 64/Yanda 24)F_1	Henan	1975	1	7+8	2+12	b	d
Jimai 6	Shijiazhuang 40/Nongda 311	Hebei	1976	N	7+9	2+12	a	b
Yannong 15	Youxuan 57/St2422/464	Shandong	1976	1	7+9	5+10	c	f
Jinan 13	Orofen/(Huixianhong/Bima 4)	Shandong	1977	1	7+9	2+12	d	b
Xian 8	Aiganzao/Stl427/506	Shaanxi	1978	1	7+9	5+10	c	g
Xiaoyan 6	St2422/464/Xiaoyan 96	Shaanxi	1979	1	14+15	2+12	d	d
Yumai 2	$65(14)_3$/Kanghuihong	Henan	1983	1	7+9	5+10	d	d
Aimengniu	Aifeng 3/(Mengxian 201/Neuzucht)F_1	Shandong	1985	1	7+8	2+12	a	j
Jimai 26	(Aiganzao/Lovrin 10)F_1/Jinfeng 1	Hebei	1986	N	7+8	2+12	c	b
Yumai 10	ПреПгорНая 2/Yanshi 4	Henan	1988	1	7+9	5+10	d	j
Jimai 32	Nongda 311/Kefan 68	Hebei	1989	N	7+9	2+12	d	j
Yumai 13	Bainong 3217/9612-2	Henan	1989	1	7+8	5+10	d	j
Lumai 14	C149/75-26	Shandong	1990	1	7+8	5+10	c	j
Yumai 18	Zhenzhou 761/Yanshi 4	Henan	1990	N	14+15	5+10	b	d
Yangmai 6	Dafeng 1087/Zao 5	Jiangsu	1991	N	7+8	2+12	a	g
Lumai 19	(7014/Zhongsu 68)F_1/$F_{16\text{-}71}$	Shandong	1992	1	7+8	2+12	a	d
Yumai 21	(Bainong 791/Yumai 2)/(Lumai1/Yanshi 4)	Henan	1992	N	7+9	2+12	d	j
Shanyou 229	7853/80356-10	Shaanxi	1993	1	14+15	5+10	c	j
Fuyang 936	(Wanmai20/Jimai 5418)/(Neixiang 184)	Anhui	1994	N	7+9	5+10	b	d
Jinan 17	Linfen 5064/Lumai 13	Shandong	1994	1	7+8	5+10	a	f
Jimai 38	4001/Shi 4212 — 10	Hebei	1994	1	14+15	2+12	d	j
Jimai 36	[(71507/ПреПгорНая 2)/75-78]F4/94354	Hebei	1995	N	14+15	2+12	d	j
Jinmai 47	12057/(522×K37-20)	Shanxi	1995	N	7+9	5+10	d	d
Jinmai 54	12057/(Han 522/K37-20)	Shanxi	1996	N	7+9	5+10	d	h
Shan 160	Shan 213/167 - 6 - 4	Shaanxi	1996	1	7+8	2+12	b	d
Yumai 41	394A/Yumai 2	Henan	1996	1	7+9	5+10	b	h
Yumai 47	Yumai 2/Baiquan 3199	Henan	1997	1	7+8	2+12	a	d
Gan 4589	Gan 86-4023/85Zhong47	Hebei	1998	1	7+8	2+12	d	j
Yumai 54	Bainong8717/$86(30)_1$	Henan	1998	1	7+9	5+10	a	d
Huaimai 18	Zhengzhou 891/Yan 1604	Jiangsu	1999	N	7+9	4+12	c	b

Table 1. (Cont'd.).

Variety	Crosses	Source	Breeding or introduce date	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3
Jinmai 61	Zhengzhou891/Yan 1604	Shanxi	1999	1	7+8	2+12	a	j
Youmai 2	PH85-115-2/(79401/Lumai 11)	Shandong	2000	1	14+15	2+12	d	h
Yumai 66	MZALenod Beer/Yumai 2	Henan	2000	1	7+9	5+10	a	j
Longmai 157	85(Jia)1-3/Pingliang 41	Gansu	2001	1	7+8	3+12	a	f
Xinmai 11	Beiquan 30473/Neixiang 82C6	Henan	2001	1	7+9	5+10	a	d
Yangmai 12	Yangmai 158/[(TP114/Yangmai 5)/85-853]	Jiangsu	2001	N	7+8	2+12	a	f
Zhengzhou 9023	{(Xiaoyan 6/Xinong 65)/ [83(2)3-3/84(14)43]}F3/Shan 213	Henan	2001	N	7+8	2+12	b	d
Huaimai 20	Yumai 13/Lumai 14	Jiangsu	2002	1	7+8	5+10	d	j
Yannong 21	Yan 1933/Shan 82-29	Shandong	2002	1	17+18	5+10	c	h
Xinong 979	Xinong 2611/(918/95Xuan1)F_1	Shanxi	2003	1	7+8	2+12	b	d
Yanzhan 4110	[(C39/Xibei 78(6)9-2)/ (FR81-3/Yumai 18)]/Yumai 18	Henan	2003	N	14+15	5+10	b	d
Zhoumai 16	Yumai 21/Zhou 8425B	Henan	2003	N	7+9	2+12	a	j
Hengguan 35	84Guan749/Heng 87-4263	Hebei	2004	N	7+9	2+12	f	j
Zhoumai 18	Neixiang 185/Yumai 21	Henan	2004	1	7+9	2+12	a	j
Aikang 58	(Zhoumai 11/Yumai 49)/Zhengzhou 8960	Henan	2005	1	7+9	5+10	d	d
Xiaoyan 81	Xiaoyan 54/8602	Hebei	2005	1	14+15	2+12	d	d
Yunong 9901	Xiangyangdasui/Yunong 8923	Henan	2005	1	7+9	2+12	d	j
Jimai 20	Lumai 14/Lu 884187	Shandong	2006	1	7+8	5+10	c	d
Xinmai 3306	Yumai 2/(Zhengzhou 891/Neixiang 82c6)	Henan	2006	N	7+8	2+12	a	j
Yunong 202	Yumai 21/Yunong 127	Henan	2007	N	7	2+12	d	j

All the surveyed cultivars and advanced lines were planted at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University during the 2007-2008 cropping season, according to local management practices. An augmented design was adopted in the field trial, comprising three repeats for each cultivar. Each plot consisted of four 200 cm-long rows with 23 cm between neighboring rows and 10 cm between neighboring plants. Both exotic and Chinese cultivars grew very well and no lodging was present in the trial.

Trait evaluation: During the middle of the grain filling term, 10 plants were randomly selected in each plot to determine the spikes plant^{-1}, plant height, spike length, spikelet number plant^{-1}, flag leaf length, flag leaf width, and the first internode length from the top. Grain numbers spike^{-1}, grain weight plant^{-1}, grain weight spike^{-1}, and test weight were determined after harvesting.

Flag leaf area = Flag leaf length × Flag leaf width × 0.75 (Fu *et al.*, 2001)

Quality testing: The surveyed wheat cultivars were milled into flour by a Quadrumat Junior Mill (Brabender Inc.) according to method of AACC26-20 (Anon., 1995). Flour protein and moisture contents (14% wet base) were tested by NIRS 5000 (Foss Inc., Switzerland) according to the method of AACC 39-10A (Anon., 1983). Flour water absorption, dough development time, dough stability time, and softness were tested by Fanograph (Brabender Inc.) according to AACC 44-15A (Anon., 1983). Wet-gluten content was tested according to the ICC method by the Gluten Index (Perten Inc.) and the results were modified at the level of 14% flour moisture content. A test of falling number indicated that all the surveyed cultivars had no spike sprout.

Identification of HMW-GS and LMW-GS: From the 215 cultivars, 186 were selected to identify the HMW-GS and LMW-GS by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Liu *et al.,* (2004). HMW-GS and the LMW-GS were classified using the nomenclature of Payne and Lawrence (1983), and Gupta & Shepherd (1990a), respectively. The wheat cultivars of Chinese Spring (N, 7+8, 2+12, GluA3a, GluB3a), Paron (2*, 17+18, 5+10, GluA3b, GluB3h), Opata (2*, 13+16, 2+12, GluA3b, GluB3d), Yuandong 107 (1, 7+9, 5+10, GluA3a, GluB3j) and Shanyou 150 (1, 7+8, 2+12, GluA3a, GluB3b) were used as control in each run to ensure the accurate bands type in SDS-PAGE. Allelic variations at the Glu-D3 locus could not be identified by this procedure. The allelic diversities of HMW-GS and LMW-GS are listed in Table 1.

Statistical analysis: Surveyed cultivars were classified into 10 different levels from first $[X_i < \bar{X} - 2S]$ to tenth $[X_i < \bar{X} + 2S]$ according to their averages ($\bar{X}$) and standards deviation (S) and every 0.5S was a level. The relative frequency of every level was used to calculate the diversity index.

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

P_i is the percentage of the sample number within the level in the surveyed total numbers, according to Shannon and Weaver (1949). Software SPSS (Statistical Product and Service solutions) 16.0 was used for statistics analysis.

Results

Evolution of agronomic and quality traits: The mean, variation coefficient, and diversity index of agronomic and quality traits of the wheat cultivars from different periods are shown in Table 2. From the first stage to the fourth stage, the averages of the 8 surveyed agronomic characteristics significantly increased, including grain numbers spike^{-1}, grain weight spike^{-1}, 1000-grain weight, grains weight plant^{-1}, spikelet spike^{-1}, sterile spikelet spike^{-1}, flag leaf width, and flag leaf area. The average grain spike^{-1} apparently increased from 46.37 in the first stage to 51.85 in the fourth stage, and 1000-grain weight sharply increased from 31.67 g in the first stage to 41.63 g in the fourth stage. However, the averages of the other 4 agronomic and 2 quality characteristics significantly decreased, including spikes plant^{-1}, plant height, first internode length from the top, flag leaf length, protein content, and wet gluten content. In particular, the average plant height dramatically decreased from 112.70 cm in the first stage to 83.99 cm in the fourth stage. Moreover, changes of spike length and growth period fluctuated from decrease to increase in the four stages. Test weight, flour water absorption, dough development time, dough stability time, and softness showed no significant changes. In addition, 1000-grain weight and grain numbers spike^{-1} increased by 3.22 g, 6.22 g, and 0.52 g, and -1.24, 1.80, and 4.92 between every 2 neighboring stages, respectively. Spikes plant^{-1} and plant height decreased by 2.23, 5.60, and 0.73, and 10.86 cm, 17.03 cm, and 0.82 cm between every 2 neighboring stages, respectively. The diversification of agronomic and quality traits in Table 2 indicated that the wheat breeding objectives had concentrated on decreasing plant height and increasing grain weight before the 1990s and then changed to increasing grain numbers spike^{-1} and 1000-grain weight.

Table 2. Average, variation coefficient and diversity index of agronomic and quality traits in four different periods.

Traits	Mean				CV (%)				Diversity index (H´)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Grain spike^{-1}	46.37 b	45.13 b	46.93 b	51.85 a	23.41	18.95	19.26	18.81	1.815	1.995	2.096	1.987
Grain weight spike^{-1} (g)	1.63 c	1.73 c	2.09 b	2.32 a	27.02	28.76	22.54	22.00	1.867	1.917	2.033	2.000
1000-grain weight (g)	31.67 c	34.89 b	41.11 a	41.63 a	23.77	18.53	16.69	19.41	1.841	1.977	2.056	2.062
Grain weight plant^{-1} (g)	22.17 b	23.26 ab	24.18 ab	25.64 a	26.36	32.39	26.71	27.41	1.996	2.017	2.073	2.008
Spikes plant^{-1}	25.23 a	23.00 b	17.40 c	16.67 c	26.32	25.59	18.38	14.97	1.846	1.775	2.078	1.907
Spikelet spike^{-1}	20.58 b	20.55 b	21.2 b	21.96 a	8.58	8.91	6.35	7.87	2.028	1.959	2.06	1.946
Sterile Spikelet spike^{-1}	1.60 b	1.75 b	2.13 a	1.96 ab	30.07	51.76	39.19	40.35	1.852	1.900	2.054	1.857
Spike length (cm)	10.26 a	9.60 b	10.30 a	10.63 a	15.44	15.43	15.09	11.57	1.937	2.014	1.972	2.001
Growth period (d)	223.79 a	223.45 a	222.39 b	223.4 a	1.32	0.87	0.86	0.84	1.921	1.611	1.885	1.875
Plant height (cm)	112.70 a	101.84 b	84.81 c	83.99 c	13.73	14.53	10.02	14.5	1.947	1.811	2.008	1.708
The first internode length from top (cm)	33.79 a	31.87 a	26.26 b	26.40 b	19.88	16.39	13.2	16.41	1.885	1.918	2.071	1.892
Flag leaf width (cm)	1.52 c	1.65 b	1.82 a	1.91 a	14.68	15.82	11.91	10.42	2.05	2.003	2.064	1.855
Flag leaf length (cm)	22.05 a	20.80 ab	19.76 b	20.20 b	13.91	16.35	13.82	14.73	1.972	1.85	2.06	1.862
Flag leaf area (cm^2)	25.30 b	26.01 b	27.15 ab	28.95 a	22.15	27.17	22.51	19.94	1.963	1.994	2.032	1.807
Test weight (g/L)	800.46 a	800.17 a	804.81 a	799.00 a	2.21	2.5	2.35	3.02	1.975	1.873	1.988	1.876
Protein content	14.55 a	14.04 ab	13.56 b	13.93 ab	9.87	11.28	10.4	7.83	1.992	1.919	2.045	1.994
Flour water absorption	62.66a	63.38a	63.66a	63.85a	5.27	4.40	6.02	4.64	1.835	2.026	2.093	1.988
Wet gluten content	30.62 a	29.24 ab	28.39 b	29.34 ab	12.13	12.46	11.94	11.23	1.881	1.857	2.028	1.981
Dough development time (min)	5.67a	5.22a	5.16a	5.00a	56.71	60.45	49.06	43.99	1.712	1.548	1.886	1.776
Dough stability time(min)	9.57a	8.92a	7.45a	7.38a	69.96	72.40	71.96	73.65	1.714	1.534	1.780	1.488
Softness(FU)	77.83a	71.17a	82.20a	80.63a	71.27	64.62	53.44	53.04	1.874	1.896	1.982	1.974

Note: Means in the same raw followed by different letters indicate significant differences at the 0.05 probability level

The diversity index of spikes plant^{-1}, growth period, development time, stability period, and plant height showed great differences between wheat cultivars in the 4 periods. However, the diversity index of grain weight plant^{-1}, spikelet spike^{-1}, spike length, and protein content changed little. The average diversity indexes of the 4 periods among the 14 agronomic traits were 1.923, 1.910, 2.039, and 1.912, respectively, while the averages between the 7 quality traits were 1.855, 1.808, 1.972, and 1.868, respectively. The results demonstrated that the diversity indexes of the agronomic traits were higher than those of the quality traits in the same periods. Evaluating the extent of genetic variation of the diversity index for 21 traits in different periods indicated that the third period had the highest average value (2.016) and the second period had the lowest average value (1.876). The first period with 1.900 was higher than the fourth period (1.897). Genetic diversity showed a decreasing - increasing - decreasing tendency from a chronological point of view.

Evolution of HMW-GS and LMW-GS: The HMW-GS and LMW-GS profiles in the four different periods are shown in Table 3. The HMW-GS and LMW-GS had 17 and 12 allelic variations in the germplasm resources of the Huanghuai wheat area, respectively. Surprisingly, a novel HMW-GS combination 20/8 never reported before was identified in 1B chromosome of Chinese landrace cultivar Heputou (Fig. 1 of lane 2). There were no significant differences in term of the quantity of HMW-GS and

LMW-GS allelic variation in the four periods. The allelic variations of HMW-GS in periods I, II, III, and IV were 12, 9, 12 and 11, respectively, while there was no obvious difference in LMW-GS. Among the cultivars, the frequencies of subunits 1, 7+9, 14+15, 5+10, GluA3d, and GluB3j gradually increased, and the frequencies of subunits N, 7+8, 2+12, GluA3a, and GluA3f, gradually decreased. The frequencies of subunits 2*, 17+18, GluA3c, GluB3d, GluB3f, and GluB3g fluctuated between increase and decrease. Subunits 22, 20/8, 2.2+12, and GluB3a were detected in the cultivars before the 1960s, and subunits 6+8, 13+16, 3+12, and 4+12 subunits were detected in the cultivars after the 1980s. The predominant subunits in the cultivars before the 1980s were N, 1, 7+8, 2+12, GluA3a, and GluB3d. After the 1980s, the predominant subunits were 1, 7+9, 2+12, 5+10, GluA3d, and GluB3j. The frequencies of subunits 1, 14+15, 5+10, and GluA3d in the period IV increased by 37.12%, 7.81%, 19.98%, and 18.56%, respectively, compared with those in the period I, while subunits 2*, 7+8 and GluB3d decreased by 14.30%, 30.23%, and 15.93%, respectively. The frequencies of subunit 7+9 and GluB3j increased by 26.88% and 34.28%, respectively, while the frequencies of N, 2+12 and GluA3a decreased by 22.82%, 22.41%, and 11.97%, respectively.

Table 3. Allelic variation and frequency of HMW-GS and LMW-GS in wheat varieties of different periods.

Gene locus	Subunit	Frequency (%)			
		I	II	III	IV
Glu-A1	1	27.59	45.45	56.67	64.71
	2*	17.24	18.18	6.67	2.94
	N	55.17	36.36	36.67	32.35
Glu-B1	7	3.45	0.00	0.00	2.94
	7+8	65.52	48.48	28.89	35.29
	7+9	17.24	33.33	50.00	44.12
	6+8	0.00	0.00	2.22	0.00
	13+16	0.00	0.00	1.11	0.00
	14+15	6.9	12.12	16.67	14.71
	17+18	0.00	6.06	1.11	2.94
	22	3.45	0.00	0.00	0.00
	20/8 [a]	3.45	0.00	0.00	0.00
Glu-D1	2+12	72.41	66.67	57.78	50.00
	2.2+12	3.45	0.00	0.00	0.00
	3+12	0.00	0.00	0.00	5.88
	4+12	0.00	0.00	1.11	0.00
	5+10	24.14	33.33	41.11	44.12
Glu-A3	GluA3a	41.38	27.27	27.78	29.41
	GluA3b	20.69	18.18	12.22	17.65
	GluA3c	17.24	21.21	18.89	14.71
	GluA3d	13.79	27.27	38.89	32.35
	GluA3f	6.90	6.06	2.22	5.88
Glu-B3	GluB3a	3.45	0.00	0.00	0.00
	GluB3b	10.34	6.06	10.00	5.88
	GluB3d	48.28	51.52	28.89	32.35
	GluB3f	13.79	15.15	8.89	11.76
	GluB3g	6.90	15.15	5.56	2.94
	GluB3h	10.34	3.03	6.67	5.88
	GluB3j	6.90	9.09	40.00	41.18

[a] 20/8 indicated that 20 and 8 subunits appeared simultaneously on chromosome 1B.

The HMW-GS and LMW-GS component type and frequency of the wheat cultivars during the different periods are presented in Table 4. The HMW-GS and LMW-GS component type of the germplasm resources showed an increasing-decreasing tendency during the four periods, and the predominant component type of the germplasm resources during the different periods showed obvious differences. The predominant component types of HMW-GS and LMW-GS were N, 7+8, 2+12, and combination of GluA3b, GluB3d, GluA3d in wheat cultivars before the 1980s. After the 1980s, the predominant component type of HMW-GS and LMW-GS were 1, 7+9, 5+10, 7+8, and GluA3a, GluB3j, GluA3d in the cultivars.

The usage of wheat germplasm resources in Huanghuai area: The pedigree analysis of cultivars from the different periods was shown in Table 5. During the first period, the parents mainly came from abroad and local accessions in China. During the second period, parents were introduced from abroad, the percentage of local landraces used significantly declined, and the percentage of released cultivars used significantly increased. In period II, the prevalent parents were mainly alien accessions, including Funo, Abbondanza, St2422/464, Early Premium, Orofen, Mentana, Triumph, and released cultivars such as Xiannong 39, Neixiang 5, Xinong 6028, Bima 4, and Fengchan 3. During the third period, the prevalent parents mainly came from the released cultivars from the former period or germplasm created by distant hybridization. Though the cultivars introduced from abroad had been fully utilized, the patterns of parents used changed. In the first and second periods, hybrid breeding was mainly performed among cultivars of alien cultivars and released cultivars. In the third period, alien cultivars were crossed with one local variety, and then their offspring were crossed with another local variety for hybrid breeding. Alternatively, alien cultivars were crossed with one local variety for traits improvement, and then the improved variety was then crossed with the local variety for hybrid breeding. The cultivars mainly utilized in the third period were Bainong 3217, Yumai 2, Zhou 8425, Yumai 18, Yumai 21, and Aimengniu. In fourth period, the parents used were mainly released cultivars from the former period, such as Yumai 18, Yumai 21, and Lumai 14. The breeding objective was not focused on new quality germplasm resources.

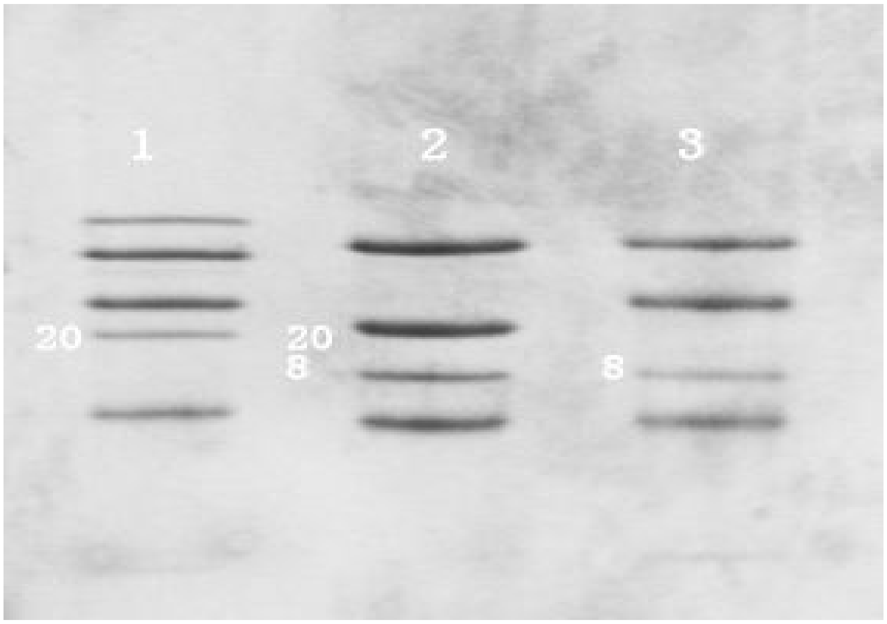

Fig. 1. A novel HMW-GS combination 20/8 (lane 2) was identified in Chinese landrace Heputou (lane 2) by SDS-PAGE (13%). Wheat varieties were used as control. Lane 1, Wanmai 33; Lane 3, Chinese Spring.

Table 4. The HMW-GS and LMW-GS component type and frequency in different periods.

Component type of HMW-GS			Frequency (%)				Component type of LMW-GS		Frequency (%)			
			I	II	III	IV			I	II	III	IV
N	7	2+12	3.45	0.00	0.00	3.45	*GluA3a*	*GluB3b*	10.34	3.03	7.78	2.94
N	22	2+12	3.45	0.00	0.00	0.00	*GluA3a*	*GluB3d*	10.34	12.12	0.00	2.94
N	14+15	5+10	3.45	3.13	3.33	6.90	*GluA3a*	*GluB3f*	6.90	3.03	3.33	8.82
N	14+15	2+12	0.00	3.13	3.33	0.00	*GluA3a*	*GluB3g*	0.00	3.03	3.33	0.00
N	20/8	2+12	3.45	0.00	0.00	0.00	*GluA3a*	*GluB3h*	6.90	0.00	1.11	0.00
N	6+8	2+12	0.00	0.00	1.11	0.00	*GluA3a*	*GluB3j*	6.90	6.06	12.22	14.71
N	7+8	2+12	31.03	21.88	7.78	10.34	*GluA3b*	*GluB3b*	0.00	0.00	1.11	0.00
N	7+8	2.2+12	3.45	0.00	0.00	0.00	*GluA3b*	*GluB3d*	17.24	15.15	10.00	17.65
N	7+8	5+10	0.00	3.13	2.22	0.00	*GluA3b*	*GluB3g*	3.45	0.00	0.00	0.00
N	7+9	2+12	3.45	6.25	8.89	13.79	*GluA3b*	*GluB3h*	0.00	3.03	1.11	0.00
N	7+9	4+12	0.00	0.00	1.11	0.00	*GluA3c*	*GluB3b*	0.00	0.00	4.44	0.00
N	7+9	5+10	3.45	0.00	8.89	3.45	*GluA3c*	*GluB3d*	10.34	9.09	2.22	2.94
1	13+16	5+10	0.00	0.00	1.11	0.00	*GluA3c*	*GluB3f*	3.45	6.06	1.11	0.00
1	14+15	2+12	3.45	3.13	7.78	6.9	*GluA3c*	*GluB3g*	3.45	3.03	2.22	0.00
1	14+15	5+10	0.00	3.13	1.112.	3.45	*GluA3c*	*GluB3h*	0.00	0.00	2.22	2.94
1	6+8	2+12	0.00	0.00	1.11	0.00	*GluA3c*	*GluB3j*	0.00	3.03	6.67	8.82
1	7+8	2+12	17.24	9.38	12.22	3.45	*GluA3d*	*GluB3b*	0.00	3.03	2.22	2.94
1	7+8	3+12	0.00	0.00	0.00	6.90	*GluA3d*	*GluB3d*	6.90	15.15	8.89	8.82
1	7+8	5+10	3.45	3.13	5.56	20.69	*GluA3d*	*GluB3f*	3.45	6.06	4.44	2.94
1	7+9	5+10	3.45	12.50	15.56	13.79	*GluA3d*	*GluB3g*	0.00	3.03	0.00	2.94
1	7+9	2+12	0.00	9.38	10	17.24	*GluA3d*	*GluB3h*	3.45	0.00	2.22	0.00
1	17+18	2+12	0.00	6.25	0.00	0.00	*GluA3d*	*GluB3j*	0.00	0.00	21.11	14.71
1	17+18	5+10	0.00	0.00	1.11	3.45	*GluA3f*	*GluB3a*	3.45	0.00	0.00	0.00
2*	7+8	2+12	3.45	6.25	0.00	0.00	*GluA3f*	*GluB3b*	0.00	0.00	2.22	0.00
2*	7+8	5+10	6.90	6.25	0.00	3.45	*GluA3f*	*GluB3d*	3.45	0.00	0.00	0.00
2*	7+9	5+10	3.45	3.13	1.11	0.00	*GluA3f*	*GluB3g*	0.00	6.06	0.00	0.00
2*	7+9	2+12	3.45	3.13	5.56	3.45	*GluA3f*	*GluB3h*	0.00	0.00	0.00	2.94
							GluA3f	*GluB3j*	0.00	0.00	0.00	2.94

Table 5. The source of parents of wheat varieties in the Huanghuai wheat region.

Period	No.	Cultivars whose pedigree contained alien wheat		Cultivars whose pedigree contained landrace varieties		Cultivars whose pedigree contained released wheat		Varieties bred by distant hybridization	
		No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)
I	8	7	87.50	3	37.50	0	0.00	0	0.00
II	29	18	62.07	4	13.79	8	27.59	0	0.00
III	68	13	19.12	2	2.94	50	73.53	4	5.88
IV	61	0	0.00	0	0.00	60	98.36	1	1.64

Discussion

The evolution of germplasm resources has accompanied the continuous progress in wheat breeding. From the results of this study, it can be concluded that wheat traits have been greatly improved by breeding during the last 60 years in the Chinese Hanghuai wheat region. The grain weight spike^{-1}, 1000-grain weight, grain weight plant^{-1} and flag leaf area of cultivars, compared with those of cultivars before the 1960s, were significantly increased. The spikes plant^{-1} and plant height both decreased. After the 1980s, breeders concentrated on improving wheat quality and developed a lot of wheat cultivars with strong-gluten or weak-gluten. Since that time, the quality characters of wet gluten content, protein content and dough stability time in wheat cultivars have decreased compared with earlier times. However, the number of wheat cultivars with strong-gluten or weak-gluten has increased. Based on previous reports (Payne & Lawrence 1983; Trethowan 2001; Luo *et al.,* 2001; Liu *et al.,* 2004 and 2005), some subunits, including 1 and 2* at the Glu-A1 locus; 7+8, 17+18 and 14+15 at the Glu-B1 locus; 5+10 at the Glu-D1 locus; GluA3d and GluB3d, have an apparently positive effect on wheat processing quality. Other subunits, including N (Glu-A1 locus missing), 7+9 at the Glu-B1 locus, 2+12 at the Glu-D1 locus, GluA3a, and GluB3j, have a significant negative effect on wheat processing quality. Two major classes of glutenin polypeptides have been identified in wheat endosperm, designated as HMW-GS and LMW-GS; both classes occur in flour as cross-linked proteins resulting from inter-polypeptide disulfide linkages. As for glutenin subunit diversity, alleles encoding subunits 2*, 7+8, and GluB3d were mostly available before the 1980s; thereafter alleles encoding subunits 1, 14+15, 5+10 and GluA3d appeared. From periodⅠ to period Ⅳ, the frequencies of subunits 7+9, and GluB3j gradually increased, and the frequencies of subunits N, 2+12, and GluA3a gradually decreased. The HMW-GS and LMW-GS subunit type showed an increasing-decreasing tendency and the predominant subunit type of the wheat germplasm resources in the four different periods were different. We can concluded that the frenqency and type of HMW-GS and LMW-GS in Huanghuai wheat cultivars changed greatly.

Although previous studies on the relationship between the crop improvement and germplasm diversity reported conflicting results, the majority concluded that crop improvement had significant effects on the germplasm diversity (Wright 1965, Walsh 1981, Tranquilli *et al.,* 2000, Hao *et al.,* 2006, and Routray *et al.,* 2007). Our results showed that the germplasm diversity in Huanghuai wheat area had fluctuated during the process of breeding. The germplasm diversity narrowed during the period 1961-1980 compared with the years before 1961. This might have resulted from using a narrow range of parents in the wheat cultivars breeding process. The parents were mainly a few foreign cultivars, local accessions, and cultivars released during an earlier period, such as Early Premium, Triumph, Funo, Abbondanza, Orofen, Mentana, Huixianhong, Bima 4, Fengchan 1, Fengchan 3, Xinong 6028, Xiannong 39, and Neixiang 5. As a result, the breeding cultivars had relatively close genetic relationships, and the genetic diversity of wheat cultivars in this period was very narrow. A number of new germplasm resources were created by distant hybridization, and by physical and chemical mutagenesis. The wheat variety Xiaoyan 6 was created by crossing bread wheat with agropyron elongatum Xiaoyan 96 (Jin *et al.,* 1986). The new wheat variety Zhou 8425 and Yumai 66 were obtained by crossing bread wheat with Triticale (Zhang *et al.,* 2000). A new wheat germplasm, Aimengniu, was cultivated using Neuzucht containing the rye Petkus1R gene (Qi *et al.,* 2001). Taking full advantage of the wheat germplasm containing foreign genes has greatly enriched the genetic background. Thus the genetic diversity in this period was significantly higher than that in the former period.

The genetic diversity of wheat germplasm resources in the period 2001-2007 was significantly lower than that of the period 1981-2000. This is probably because the parents of the germplasm resources came from the cultivars of the former period. The foreign cultivars and local accessions had not been fully used and the new germplasms with distant phylogenetic relationships were not fully exploited. If breeding activities were limited to using existing germplasm resources, rather than focus on creating new germplasm, genetic diversity would decrease after a period of time. Otherwise, if more attention had been paid to innovation and introduction of new germplasms, the genetic diversity would have increased.

Elite wheat germplasm has been fully introduced in wheat breeding. Using the introduced cultivars as parents, including Lovrin 10, Orofen, Abbondanz, Shanqian 2, Funo, Mentana, Кавказ, St2422/464, Early Premium and Quality, 312, 253, 237, 200, 188, 113, 94, 59, 59 and 46 cultivars were registered before 1997, respectively (Dong and Zheng, 2000). Using the landrace cultivars Mazhamai, Xinong 60, Jiangdongmen, and Yanda 1817 as parents, 196, 30, 50, and 23 cultivars was developed, respectively (Jin, 1983). Using the cultivars Yumai 2, Aimengniu, and Lumai 14 as parents, 43, 18, and 19 cultivars, respectively, were cultivated in Huanghuai wheat area after the 1980's. The frequent use of elite germplasm has resulted in the development of a large number of excellent cultivars, which have made a tremendous contribution to wheat production; however at the same time, the genetic distance between cultivars has narrowed and the genetic diversity of germplasm resources has been reduced.

It could be concluded that local cultivars have not been fully utilized since the 1980s. The foreign and local accessions were not thoroughly introduced, and the slow progress of the innovation of new germplasm has made it difficult to further enhance breeding during the early 21st century. China has more than 13,000 landraces, among which many useful genes have not been exploited. In addition, useful genes in germplasm resources of American and Canadian cultivars have not been fully utilized. We should further explore useful genes from local landraces or strengthen the introduction, identification, and utilization of foreign germplasm resources to widen the genetic diversity. Meanwhile, we can take advantage of useful genes for promoting the productivity of breeding cultivars by using modern biotechnology to transfer useful genes from different species into bread wheat, creating new germplasm resources and broadening the genetic basis of the existing germplasm resources.

Acknowledgments

We thank Yang Zhou (Chinese Academy of Agricultural Sciences) and Tian-Min Shen (Henan wheat breeding international research center) for sending the seeds of wheat accessions. This work was supported by the grants from the Key Projects in the National Science & Technology Pillar Program during the Twelfth Five-Year Plan Period (2011BAD07B01), the National "973" Foundation of China (2009CB118300) and the Important Public Welfare Project of Henan Province.

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(Received for publication 28 November 2009)