

# Genetic characterization and evaluation of twenty Chinese winter wheat cultivars as potential sources of new diversity for breeding

JIŘÍ HERMUTH, LEONA LEIŠOVÁ-SVOBODOVÁ, JANA BRADOVÁ, KLÁRA KOSOVÁ\*,  
VÁCLAV DVOŘÁČEK, ILJA TOM PRÁŠIL, LADISLAV DOTLAČIL

Crop Research Institute (CRI), Prague-Ruzyně, Czech Republic

\*Corresponding author: [kosova@vurv.cz](mailto:kosova@vurv.cz)

**Citation:** Hermuth J., Leišová-Svobodová L., Bradová J., Kosová K., Dvořáček V., Prášil I.T., Dotlačil L. (2019): Genetic characterization and evaluation of twenty Chinese winter wheat cultivars as potential sources of new diversity for breeding. Czech J. Genet. Plant Breed., 55: 8–14.

**Abstract:** Twenty Chinese and two Czech wheat cultivars were assessed with respect to their genetic diversity using single sequence repeat (SSR) markers and high-molecular-weight (HMW)-glutenin alleles. Phenotypic characteristics including morphological, productivity and quality characters, earliness, and resistance to stresses were evaluated in field experiments and, consequently, drought tolerance (estimated by means of  $^{13}\text{C}$  discrimination method) and winter hardiness (in provocation test) were tested. Employment of both genetic and phenotypic characteristics allowed identifying promising sources of new genetic diversity. Some Chinese cultivars were considered as potential donors of quality characters, earliness or drought tolerance. The results indicate that new genetic diversity can be found in resources of geographically distant origin.

**Keywords:** agronomical characters; donors; genetic diversity; markers; stress tolerance; *Triticum aestivum*

World agriculture is facing increasing demands on production and its quality, as well as adaptation to climate changes. Since further intensification of technologies and use of agrochemicals are limited due to their negative impacts on environment and health, genetic improvement of crops gains importance. Breeding can accomplish this mission by employment of advanced technologies and utilization of new genetic diversity. Needs for a new genetic diversity and a broadening of genetic base of modern cultivars are often discussed in many crops, including wheat (ARAUS *et al.* 2007; CATTIVELLI *et al.* 2008). In wheat, we can search for new diversity (beside wild relatives and landraces) in genetically distinct cultivars, e.g. from geographically remote areas. In our study, we utilized Chinese wheat cultivars originating from the main wheat growing zone II. „Yellow and Huai River Valleys Facultative Region“ in China wheat production (HE *et al.* 2001).

The zone has temperate climate with occurring low temperatures, frost and drought periods. One of the selected cultivars, Xiaoyan 6, was the most widely grown cultivar in 1980s. It bears some genes transferred from *Agropyron elongatum* conferring disease resistance. Ningchun 16 was derived from Ningchun 4 in Ningxia province. It is resistant to drought, leaf rust and powdery mildew and became one of the leading varieties in Inner Mongolia, Xinjiang, and Ningxia with acreage of 80 000 ha in 1990s. Detailed information about these varieties can be found in HE *et al.* (2001).

Genotyping represents a promising approach to evaluate a level of diversity within a crop species. For this purpose several methods are used. Analysis of microsatellite loci (single sequence repeat, SSR) is often used for many advantages. In wheat, microsatellite analysis was used for diversity studies and for QTL analysis of many different features

<https://doi.org/10.17221/192/2017-CJGPB>

(PLASCHKE *et al.* 1995; AKKAYA & BUYUKUNAL-BAL 2004; SONG *et al.* 2005). In wheat, also grain storage proteins, mainly glutenins and gliadins, can be used for cultivar characterisation and as markers of quality characters. Especially, the high-molecular-weight glutenin subunits (HMW-GS) are considered to be significantly related to the technological quality of common wheat (BRANLARD *et al.* 2001). The knowledge of glutenin-subunits composition is valuable for evaluation of intra- and/or inter-varietal polymorphism and so for predicting dough quality in wheat (ZHELEVA *et al.* 2007).

Due to climate change, plant characters associated with earliness and stress tolerance attain increasing importance. However, field or pot tests of drought tolerance are time-consuming and expensive. Investigation of carbon isotope  $^{13}\text{C}$  discrimination by plants could be a quick and efficient method for screening potential donors in extensive collections of genetic resources. In wheat, plant water release is regulated by evapotranspiration, however, stomata serve also for  $\text{CO}_2$  uptake. In wheat as a C3 plant, stomatal openness and thus drought stress impacts can be indirectly monitored via RuBisCO  $^{13}\text{C}$  discrimination activity. The rate of  $^{13}\text{C}$  discrimination can be a convenient indicator of the transpiration efficiency in wheat (FARQUHAR & RICHARDS 1984; EHDAIE *et al.* 1991) as well as for other species (CONDON *et al.* 1993). Low  $\delta^{13}\text{C}$  values can be applied as a criterion for selection of materials with higher transpiration efficiency and stable biomass production under drought conditions (REBETZKE *et al.* 2002).

The aim of this study was to characterise genetic diversity and phenotypic characters in the set of 20 selected Chinese winter wheat cultivars and identify donors for possible further utilization in research and breeding.

## MATERIAL AND METHODS

**Material and field experiments.** Twenty promising Chinese cultivars (listed in Table 1) were selected and grown along with two Czech check cultivars (Bohemia, Bodyček) in the nursery of wheat genetic resources in Crop Research Institute (CRI), Prague-Ruzyně. The Chinese cultivars were obtained from Northwest University of Agriculture and Forestry (NWUAF), Yangling, Shaanxi, P.R. China, as a result of a bilateral collaboration. Plants were sown in field tests, carried out in locations Prague-Ruzyně (2014 and 2015) and Kroměříž (2015) in plots of 2 m<sup>2</sup>. We used standard growing technologies, but no

growth regulators and only limited N-fertilization were applied. Using National Descriptor List for Wheat we estimated winter hardiness and resistance to powdery mildew. We recorded days to flowering and maturity and measured plant height. Before harvest, 30 spikes from each plot were sampled to analyse characters of spike productivity. Crude protein and starch contents (measured on intact grains by NIR Nicolet Antaris II; Nicolet, Czech Republic) and wet gluten content in grain (evaluated by ICC No. 155 Glutomatic system; Perten Instruments, Sweden) were analysed in the Lab of Quality, CRI Prague-Ruzyně.

**DNA extraction and microsatellite analysis.** DNA was extracted from young leaves using CTAB according to the optimised protocol based on SAGHAI MAROOF *et al.* (1984). For SSR analysis, a set of 42 SSR markers

Table 1. Composition of high-molecular-weight (HMW) glutenin alleles in 20 Chinese winter wheat cultivars and two Czech check cultivars

Cultivar	Line No.	Incidence of HMW-glutenin alleles (subunits) in locus		
		<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>
Dong Ying No. 1	1	<i>c</i> (0)	<i>b</i> (7+8)	<i>a</i> (2+12)
	2	<i>c</i> (0)	<i>c</i> (7+9)	<i>a</i> (2+12)
	3	<i>a</i> (1)	<i>c</i> (7+9)	<i>h</i> (5+12)
Lu Mai No. 14	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>d</i> (5+10)
Ji Mai No. 21	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>h</i> (5+12)
Changwu 134	1	<i>a</i> (1)	<i>e</i> (20)	<i>a</i> (2+12)
Ningchun 16	1	<i>a</i> (1)	<i>c</i> (7+9)	<i>a</i> (2+12)
Zhengmai 9023	1	<i>c</i> (0)	<i>e</i> (20)	<i>h</i> (5+12)
Zhengmai 9405	1	<i>a</i> (1)	<i>e</i> (20)	<i>d</i> (5+10)
Xiaoyan 216	1	<i>a</i> (1)	<i>c</i> (7+9)	<i>a</i> (2+12)
Xiaoyan 22	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>a</i> (2+12)
Xinong 889	1	<i>c</i> (0)	<i>b</i> (7+8)	<i>a</i> (2+12)
Xinong 2000	1	<i>c</i> (0)	<i>b</i> (7+8)	<i>a</i> (2+12)
Xinong 1043	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>d</i> (5+10)
Shaannong 1186	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>a</i> (2+12)
Shaan 538	1	<i>c</i> (0)	<i>b</i> (7+8)	<i>a</i> (2+12)
Xiaoyan 986	1	<i>a</i> (1)	<i>c</i> (7+9)	<i>a</i> (2+12)
Dengfeng 168	1	<i>c</i> (0)	<i>c</i> (7+9)	<i>a</i> (2+12)
Weike 2	1	<i>c</i> (0)	<i>c</i> (7+9)	<i>a</i> (2+12)
E45	1	<i>a</i> (1)	<i>c</i> (7+9)	<i>h</i> (5+12)
	2	<i>c</i> (0)	<i>b</i> (7+8)	<i>a</i> (2+12)
Zhengnong 16	1	<i>a</i> (1)	<i>c</i> (7+9)	<i>d</i> (5+10)
W 38	1	<i>a</i> (1)	<i>b</i> (7+8)	<i>h</i> (5+12)
Bohemia (check)	1	<i>c</i> (0)	<i>i</i> (17+18)	<i>d</i> (5+10)
Bodyček (check)	1	<i>c</i> (0)	<i>c</i> (7+9)	<i>a</i> (2+12)

published in RÖDER *et al.* (1998) was selected. PCR reactions were carried out according to ROUSSEL *et al.* (2005). Products of PCR reactions were separated using capillary electrophoresis in ABI PRISM 3130 (Applied Biosystems, USA) in quadruplex configuration with internal standard LIZ500 (Life Technologies, Czech Republic). Electroforeograms were evaluated using GeneMapper software (Life Technologies). A matrix of distances between genotypes was calculated using Jaccard dissimilarity coefficient in the DARwin software (PERRIER & JACQUEMOUD-COLLET 2006). For clustering, an unweighted neighbour-joining method was used. The support for the tree branches was obtained using 2000 bootstrap re-samplings.

**Determination of grain quality proteins.** Glutenins were extracted from single crushed wheat kernels and fractionated by SDS-PAGE according to BRADOVÁ and MATĚJOVÁ (2008). The bands of HMW-glutenin subunits were read using the nomenclature described by PAYNE and LAWRENCE (1983).

**Determination of winter hardiness in provocation test.** Winter hardiness was determined during winter season 2014/2015 as percentage of plants survival using provocation pot test method as described in PRÁŠIL and ROGALEWICZ (1989). Briefly, plants were exposed to winter conditions at two levels above the ground (5 and 50 cm, respectively).

**Indirect method of drought tolerance estimation based on discrimination of  $^{13}\text{C}$  uptake by plants.** Samples of dry seeds were milled and then composition and content of elements were determined in elementary analyzer (EuroEA 3028-HT fy; Eurovector, Italy). Isotope analyses of plant materials were carried out in CRI Prague-Ruzyně, Lab for Soil and Plant Analyses. International standards PDB were used for determination of  $^{12}\text{C}$  and  $^{13}\text{C}$  isotopes.

**Statistical analysis.** The obtained data were statistically evaluated using ANOVA analysis and Tukey post-hoc test at 0.05 level (Statistica 7.0, 2007).

## RESULTS AND DISCUSSION

Results of cluster analysis based on genetic distances between 20 Chinese and two Czech cultivars (considered as genetically distant and used as check cultivars) are shown in Figure 1. The dendrogram consists of 4 clusters; Cluster A and cluster C can be subdivided into 2 and 4 sub-clusters, respectively. Cultivar Weike 2 was shown to be genetically distant to all other cultivars (Figure 1). Genetic similarity proved cultivars Xinong 889 and Xinong 2000 to be

identical; however, they differ in some phenotypic characteristics. The results of genotyping are supplemented by phenotypic data showing mean values of all evaluated characteristics for individual clusters and sub-clusters (Table 2).

Cluster A includes 8 cultivars, two of which (Zhengmai 9405 and Xiaoyan 216) could be distinguished as a sub-cluster a1 and the remaining 6 cultivars (Changwu 134, Ningchun 16, Xinong 889, Xinong 2000, Shaannong 1186, and Xiaoyan 986) as a sub-cluster a2. Cluster A can be characterised by low winter hardiness, short stem, earliness, high crude protein content (14.7%) and low  $^{13}\text{C}$  discrimina-

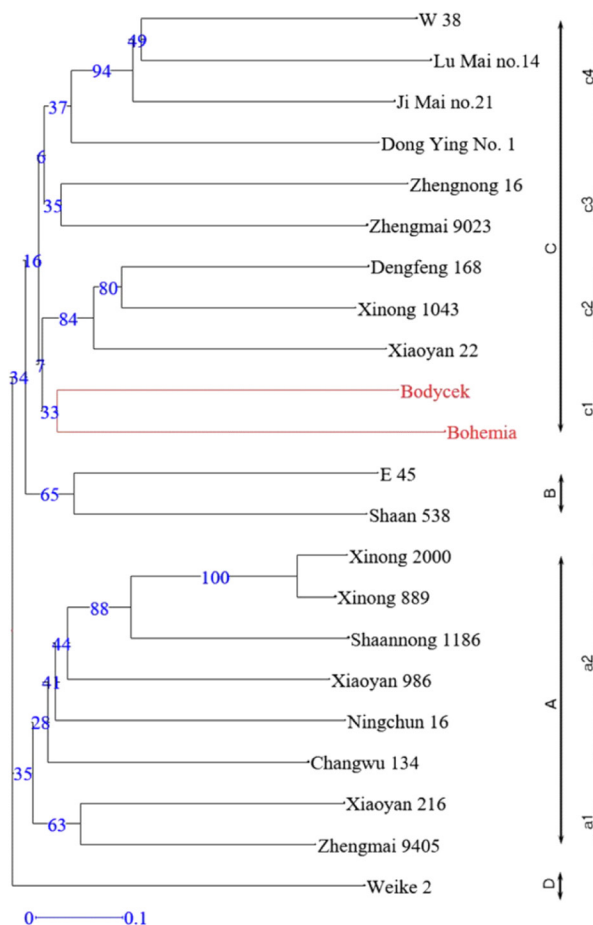


Figure 1. Dendrogram of genetic distances in the set of 20 Chinese winter wheat cultivars and two Czech cultivars (Bodyček, Bohemia; used as checks) obtained from Northwest University of Agriculture and Forestry (NWUAF), Yangling, Shaanxi, P.R. China

Distances were calculated by Jaccard dissimilarity coefficients, unweighted neighbour-joining method was used for clustering; clusters are marked by capital letter, their sub-clusters than by small letter with numeral

<https://doi.org/10.17221/192/2017-CJGPB>

Table 2. Phenotypic profiles of the clusters and sub-clusters of wheat cultivars identified by DNA microsatellite markers (SSR) and characterised by mean values of evaluated agronomical characters

Cluster/sub-cluster	Winter hardiness field*	Winter hardiness pot test**	$\delta^{13}\text{C}$ (‰)***	Plant height (cm)	Lodging*	Powdery mildew*	Maturity (days)	Grain filling period (days)	Grain No. per spikelet	Grain No. per spike	Thousand grain weight (g)	Grain mass per spike (g)	Crude protein (%)	Starch (%)	Wet gluten (%)
A ( <i>n</i> = 8)	7.1	20.9	-26.2	68.1	7.1	6.1	178	37.1	1.82	31.7	45.6	1.46	14.7	62.8	30.3
a1 ( <i>n</i> = 2)	7.3	3.5	-26.2	68.8	6.7	6.2	179	37.8	1.97	33.2	45.6	1.50	14.6	63.1	29.8
a2 ( <i>n</i> = 6)	6.9	28.3	-26.2	67.8	7.2	6.0	178	36.7	1.76	31.1	45.6	1.44	14.8	62.6	30.5
B ( <i>n</i> = 2)	8.0	59.5	-26.4	67.3	7.3	5.5	177	36.7	2.09	37.0	46.6	1.79	14.2	63.5	29.1
C ( <i>n</i> = 11)	8.0	69.1	-26.7	80.0	7.5	6.6	180	37.7	1.82	32.8	47.1	1.61	13.7	63.8	26.7
c1 ( <i>n</i> = 2)	8.3	98.5	-27.0	98.5	7.5	8.0	185	38.3	1.96	40.1	48.0	1.96	12.4	64.5	23.1
c2 ( <i>n</i> = 3)	7.9	82.7	-26.2	83.1	6.4	6.2	181	39.8	1.79	31.5	52.9	1.78	13.9	64.3	28.0
c3 ( <i>n</i> = 2)	7.3	10.0	-26.8	69.1	8.0	6.3	178	36.8	1.88	30.6	47.0	1.52	14.5	63.1	29.6
c4 ( <i>n</i> = 4)	8.3	77.8	-27.0	71.3	8.3	6.0	179	35.9	1.69	30.2	40.7	1.24	13.7	63.2	25.9
D ( <i>n</i> = 1)	7.3	7.0	-26.0	59.6	9.0	5.3	176	38.0	2.23	39.4	37.4	1.47	14.6	62.8	30.2
Mean	7.6	49.0	-26.5	73.1	7.5	6.2	179	37.3	1.85	32.9	45.8	1.54	14.1	63.3	28.4

\*Evaluation by the Czech National Descriptor List for Wheat (using scores 1–9); \*\*provocation pot test in stress conditions; \*\*\*discrimination rate of  $^{13}\text{C}$  intake by plant

tion. Sub-cluster a1 proved higher number of grains per spikelet (1.97), whereas sub-cluster a2 showed lower spike productivity. Cluster B encompasses only two short-stem early cultivars Shaan 538 and E 45, characterised by a high number of grains per spikelet (2.09) and grain weight per spike (1.79 g). Cluster C represents the largest group composed of 11 cultivars with relatively long stem, high thousand grain weight and a lower crude protein content. Two Czech cultivars Bohemia and Bodyček form sub-cluster c1 which differs from the Chinese cultivars by higher winter hardiness, higher spike productivity, and lower contents of crude protein and wet gluten (12.4% and 23.1%, respectively). Sub-cluster c2, which includes three cultivars, is characterised by relatively good winter hardiness, similar to Czech cultivars, low  $^{13}\text{C}$  discrimination, long stem, longer grain filling period (39.8 days) and good spike productivity. Two cultivars in sub-cluster c3 reveal low winter hardiness; in contrast, four cultivars in sub-cluster c4 prove good winter hardiness, but only low spike productivity and a shorter grain-filling period. Cultivar Weike 2 (cluster D) proved very high number of grains per spikelet (2.23).

Characteristics of HMW-glutenin alleles in all cultivars are shown in Table 1. Chinese cultivars Dong Jing No. 1 and E 45 consist of three and two lines, respective-

ly, other Chinese and Czech cultivars were single-lines. In 20 Chinese cultivars (Table 3) the most frequently occurring alleles were *Glu-A1a* (61%), *Glu-B1b* (48%), and *Glu-D1a* (61%). Alleles *Glu-B1e* (13%) and *Glu-D1d* (17%) can be ranked as less occurring ones. Data analysis for Chinese cultivars possessing the same HMW-glutenin alleles indicated that *Glu-B1e* allele (found in cultivars Changwu 134, Zhengmai 9023,

Table 3. Incidence of high-molecular-weight (HMW)-glutenin alleles (subunits) in 20 Chinese winter wheat cultivars representing 23 lines, and mean values of grain quality characters in the groups of cultivars bearing the same alleles

HMW-glutenin alleles (subunits)	No. of lines	Crude protein	Starch (%)	Wet gluten
<i>Glu-A1c</i> (0)	9	14.1	63.4	28.1
<i>Glu-A1a</i> (1)	14	14.7	62.8	30.4
<i>Glu-B1b</i> (7+8)	11	14.1	63.2	28.1
<i>Glu-B1c</i> (7+9)	9	14.1	63.6	28.6
<i>Glu-B1e</i> (20)	3	15.3	62.3	32.3
<i>Glu-D1a</i> (2+12)	14	14.5	63.0	29.6
<i>Glu-D1h</i> (5+12)	5	14.3	63.0	28.6
<i>Glu-D1d</i> (5+10)	4	13.8	63.7	26.9

12 Table 4. Mean values of evaluated agronomical characters in 20 Chinese and two Czech winter wheat cultivars in field experiments (Prague-Ruzyně 2014 and 2015, Kroměříž 2015) and consequent laboratory tests

Cultivar	Winter hardiness		Plant height (cm)	Lodging score	Powdery mildew*	Maturity period (days)	Grain filling period (days)	Fertile spikelets per spike	Grain No.		Thousand grain weight (g)	Grain mass per spike (g)	Crude protein (%)	Starch (%)	Wet gluten	$\delta^{13}C$ (‰)***
	field*	pot test**							per spikelet	per spike						
Dong Ying No. 1	8.0	58.0	64.8 <sup>ab</sup>	9.0	5.7 <sup>ab</sup>	177.7	37.0	18.4 <sup>cd</sup>	1.84	35.9	37.3	1.39 <sup>ab</sup>	13.9	63.0	28.0	-27.10 <sup>abcd</sup>
Lu Mai No. 14	8.7	93.0	75.9 <sup>ab</sup>	9.0	6.0 <sup>abc</sup>	179.3	34.3	15.89 <sup>abcd</sup>	1.64	27.7	43.9	1.20 <sup>a</sup>	13.2	63.5	23.2	-27.18 <sup>abc</sup>
Ji Mai No. 21	8.3	70.0	71.4 <sup>ab</sup>	9.0	6.7 <sup>abcd</sup>	179.0	36.3	16.5 <sup>abcd</sup>	1.39	25.0	41.5	1.06 <sup>a</sup>	13.5	63.1	25.1	-26.04 <sup>efgh</sup>
Changwu 134	7.3	47.0	75.8 <sup>ab</sup>	6.3	5.7 <sup>ab</sup>	176.3	34.7	16.4 <sup>abcd</sup>	1.56	27.6	41.1	1.16 <sup>a</sup>	15.6	61.6	33.9	-26.23 <sup>defgh</sup>
Ningchun 16	7.3	59.0	68.7 <sup>ab</sup>	8.3	8.3 <sup>d</sup>	180.0	35.7	18.7 <sup>d</sup>	2.02	39.2	45.6	1.89 <sup>ab</sup>	13.6	63.7	27.3	-25.67 <sup>h</sup>
Zhengmai 9023	7.0	15.0	66.1 <sup>ab</sup>	7.7	6.0 <sup>ab</sup>	177.7	36.7	16.0 <sup>abcd</sup>	1.83	30.8	43.4	1.49 <sup>ab</sup>	15.4	62.4	32.4	-26.39 <sup>bcddefgh</sup>
Zhengmai 9405	7.3	5.0	70.3 <sup>ab</sup>	6.3	6.7 <sup>abcd</sup>	180.3	39.7	15.4 <sup>abcd</sup>	1.93	30.9	47.4	1.52 <sup>ab</sup>	14.8	62.9	30.5	-26.42 <sup>bcddefgh</sup>
Xiaoyan 216	7.3	2.0	67.3 <sup>ab</sup>	7.0	5.7 <sup>ab</sup>	177.0	36.0	16.8 <sup>abcd</sup>	2.00	35.6	43.8	1.49 <sup>ab</sup>	14.3	63.3	29.1	-25.99 <sup>gh</sup>
Xiaoyan 22	7.3	80.0	77.4 <sup>ab</sup>	7.0	5.7 <sup>ab</sup>	180.0	39.7	17.0 <sup>abcd</sup>	1.76	32.5	51.7	1.83 <sup>ab</sup>	14.1	63.8	28.8	-25.64 <sup>h</sup>
Xinong 889	6.3	6.0	69.6 <sup>ab</sup>	6.7	5.3 <sup>ab</sup>	177.0	37.0	15.1 <sup>abc</sup>	1.90	30.9	47.9	1.55 <sup>ab</sup>	15.9	61.5	33.2	-26.30 <sup>cdefgh</sup>
Xinong 2000	5.3	2.0	65.7 <sup>ab</sup>	6.7	5.7 <sup>ab</sup>	178.0	38.3	14.2 <sup>a</sup>	1.59	24.4	49.0	1.15 <sup>a</sup>	15.1	61.9	32.6	-26.23 <sup>defgh</sup>
Xinong 1043	8.0	80.0	88.1 <sup>bc</sup>	6.3	7.0 <sup>bcd</sup>	181.7	40.3	16.0 <sup>abcd</sup>	1.84	31.5	56.1	1.82 <sup>ab</sup>	13.5	64.6	27.0	-26.37 <sup>bcddefgh</sup>
Shaannong 1186	8.0	37.0	63.0 <sup>ab</sup>	8.3	6.0 <sup>abc</sup>	176.0	36.3	17.3 <sup>abcd</sup>	1.61	30.8	46.9	1.47 <sup>ab</sup>	14.0	63.9	27.3	-25.79 <sup>gh</sup>
Shaan 538	8.0	73.0	68.1 <sup>ab</sup>	7.7	5.7 <sup>ab</sup>	176.7	36.7	16.6 <sup>abcd</sup>	2.14	37.5	47.8	1.81 <sup>ab</sup>	14.1	63.4	28.3	-26.83 <sup>abcdef</sup>
Xiaoyan 986	7.3	19.0	63.9 <sup>ab</sup>	7.0	5.0 <sup>a</sup>	178.3	38.3	16.9 <sup>abcd</sup>	1.90	33.5	42.9	1.43 <sup>ab</sup>	14.6	63.2	28.9	-26.75 <sup>abcdef</sup>
Dengfeng 168	8.3	88.0	83.9 <sup>abc</sup>	6.0	6.0 <sup>abc</sup>	180.3	39.3	15.8 <sup>abcd</sup>	1.79	30.6	50.8	1.69 <sup>ab</sup>	14.1	64.4	28.1	-26.59 <sup>bcddefg</sup>
Weike 2	7.3	7.0	59.6 <sup>a</sup>	9.0	5.3 <sup>ab</sup>	176.0	38.0	17.1 <sup>abcd</sup>	2.23	39.4	37.4	1.47 <sup>ab</sup>	14.6	62.8	30.2	-26.04 <sup>efgh</sup>
E 45	8.0	46.0	66.6 <sup>ab</sup>	7.0	5.3 <sup>ab</sup>	177.7	36.7	16.7 <sup>abcd</sup>	2.04	36.4	45.4	1.77 <sup>ab</sup>	14.3	63.6	29.9	-25.97 <sup>fgh</sup>
Zhengnong 16	7.7	5.0	72.1 <sup>ab</sup>	8.3	6.7 <sup>abcd</sup>	177.7	37.0	14.6 <sup>ab</sup>	1.93	30.4	50.5	1.54 <sup>ab</sup>	13.7	63.8	26.7	-27.22 <sup>ab</sup>
W 38	8.0	90.0	72.9 <sup>ab</sup>	6.3	5.7 <sup>ab</sup>	179.7	36.0	16.3 <sup>abcd</sup>	1.89	32.1	40.2	1.32 <sup>ab</sup>	14.1	63.0	27.1	-27.62 <sup>a</sup>
Bohemia (check)	8.0	99.0	110.6 <sup>c</sup>	8.3	8.3 <sup>d</sup>	187.0	39.0	18.7 <sup>d</sup>	2.10	44.3	51.5	2.32 <sup>b</sup>	12.9	64.0	24.2	-27.06 <sup>abcd</sup>
Bodyček (check)	8.7	98.0	86.4 <sup>abc</sup>	6.7	7.7 <sup>cd</sup>	183.3	37.7	17.9 <sup>bcd</sup>	1.82	35.9	44.4	1.60 <sup>ab</sup>	12.0	65.1	22.1	-26.91 <sup>abcd</sup>
Mean value	7.6	49.0	73.1	7.5	6.2	178.9	37.3	16.6	1.85	32.9	45.8	1.54	14.1	63.3	28.4	-26.47

Results were evaluated using one-way analysis of variance (ANOVA; Statistica 7.0 CZ) and Tukey post-hoc test at 0.05 level; different letters indicate significant differences at 0.05 level; \*number of days are considered from 1.1.; marking used is from National wheat classification; \*\*results of provocation pot test method expressed as percentage (%) of plant winter survival (Prague-Ruzyně, 2014/2015); cultivar Mironovskaya 808 (100% winter survival) is considered as a standard

<https://doi.org/10.17221/192/2017-CJGPB>

and Zhengmai 944405) increased the crude protein and wet gluten contents (in average by 7.7% and 11.0%, respectively). Two other genetically similar cultivars Xinong 889 and Xinong 2000 with relatively high crude protein content (15.9%) and wet gluten content (61.5%) revealed identical allelic profile *Glu-A1c*, *Glu-B1b*, and *Glu-D1a*. Allele *Glu-D1d* was found in four Chinese cultivars with lower crude protein and wet gluten contents. Similar effect was found associated with *Glu-A1c* allele.

The phenotypic data (Table 4) proved distinct characteristics in Chinese and Czech cultivars. Chinese cultivars revealed higher crude protein and wet gluten contents (on average by 15.3% and 24.8%, respectively) and no correlation to spike productivity was found. Cultivars Zhengmai 9023, Zhengmai 9405 and Xinong 889 with high crude protein content (14.8–15.9%) revealed average values of grain weight per spike (1.49–1.55 g).

Chinese cultivars were also characterised by shorter stem (70.6 cm in average) and earliness (in average, they matured by seven days earlier and grain filling period was by 1.1 day longer than in Czech cultivars). However, very early cultivars revealed grain-filling period below average. Cultivars with long grain-filling period (Zhengmai 9405, Xiaoyan 22, Xinong 1043, and Dengfeng 168) belong to slightly later ones. The major weakness of Chinese cultivars regarding their potential utilization lies in low winter hardiness, which reached in average only 45% of winter survival of Czech cultivars (in provocation test). Nevertheless, five Chinese cultivars (Lu Mai 14, Xiaoyan 22, Xinong 1043, Dengfeng 168, and W 38) reached 80% to 93% plant survival in the provocation test and from 7.3 to 8.7 in field rating. There is a large diversity in Chinese cultivars regarding the traits associated with spike productivity—from lower values in most of cultivars to some cultivars (Ningchun 16, Xiaoyan 22, Xinong 1043, and Shaan 538) showing values from 1.81 to 1.89 g grain mass per spike, which is comparable to the Czech cultivars.

Most Chinese cultivars revealed lower  $^{13}\text{C}$  discrimination values  $\delta^{13}\text{C}$  (‰) than Czech cultivars. As referred in FARQUHAR & RICHARDS 1984, low values of  $\delta^{13}\text{C}$  can indicate good drought tolerance. Values of  $\delta^{13}\text{C}$  lower than  $-26.0\text{‰}$  were found in cultivars Ji Mai 21, Ningchun 16, Xiaoyan 216, Xiaoyan 22, Shaanong 1186, Weike 2, and E 45, which can be considered as potential donors of drought tolerance. Their average value was  $-25.9\text{‰}$ , that is by 4.1% lower than in the Czech check cultivars. These cultivars proved neither genetic similarity, nor common phenotypic character-

istics. Values of  $\delta^{13}\text{C}$  proved low variation in the three experimental environments (mean value of coefficient of variation in 22 cultivars was 2.8% and only 2.0% in the above six Chinese cultivars). The differences in  $\delta^{13}\text{C}$  among cultivars were significant (Table 4).

Some of Chinese cultivars can be considered as potential donors of grain quality, but also other traits including spike productivity components, earliness, longer grain filling period and drought tolerance. Cultivars Zhengmai 9023, Zhengmai 9405, and Xinong 889 revealed high crude protein content (14.8–15.9%) and relatively good grain mass per spike, based on a higher number of grains per spikelet; their weakness is low winter hardiness. The composition of HMW-glu alleles is diverse; however, Zhengmai 9023 and Zhengmai 9405 possess *B1e* allele which has a positive effect on high crude protein content. Cultivar Xinong 889 has *A1c*, *B1b*, and *D1a* alleles. Cluster analysis indicates a genetic diversity between these three cultivars since they all belong to distinct clusters (Figure 1). In the whole set of Chinese cultivars, we proved a negative correlation between winter hardiness and crude protein content ( $r = -0.62$ ).

Earliness is another valuable character, especially when it is associated with longer grain-filling period, good spike productivity and sufficient winter hardiness. Cultivar Xinong 1043 has long grain-filling period (40.3 days), relatively good winter hardiness, and spike productivity. Similar characteristics were found also in cultivars Dengfeng 168 and Xiaoyan 22 (which proved also potentially good drought tolerance  $\delta^{13}\text{C} = -25.9\text{‰}$ ). The three mentioned cultivars are genetically related since all belong to sub-cluster c2 (Figure 1). They are relatively late in the set of Chinese cultivars; however, they are still earlier than the Czech cultivars.

Cultivars Ji Mai 21, Ningchun 16, Xiaoyan 216, Xiaoyan 22, Shaanong 1186, Weike 2, and E 45 proved low values  $\delta^{13}\text{C}$ , what indicates their possible drought tolerance. The cultivars proved neither genetic similarity nor shared phenotypic characteristics.

## CONCLUSIONS

Chinese cultivars proved high genetic diversity within the group as well as genetic distances to Czech cultivars. The clusters of genetically similar cultivars revealed common phenotypic characteristics. Determination of both genetic and phenotypic characteristics allowed us to identify sources of new genetic diversity. Some of Chinese cultivars can be considered

<https://doi.org/10.17221/192/2017-CJGPB>

as potential donors of grain quality, but also other traits including spike productivity components, earliness, longer grain filling period and drought tolerance. Cultivars Zhengmai 9023, Zhengmai 9405, and Xinong 889 revealed high crude protein content and relatively good grain mass per spike; their weakness is low winter hardiness. Cultivars Ji Mai 21, Ningchun 16, Xiaoyan 216, Xiaoyan 22, Shaannong 1186, Weike 2, and E 45 proved low values  $\delta^{13}\text{C}$ , what indicates their possible drought tolerance. The results indicate that new sources of genetic diversity can be found in genotypes of geographically distant origin.

**Acknowledgements.** The research was supported by the Czech Ministry of Education, Youth and Sports, in the scope of programme CONTACT, Project LH12158 „Diversity in rapeseed and wheat genetic resources of Chinese and Czech origin, characterization and evaluation of valuable materials using biotechnological methods for their further use“. This work was also supported by the Ministry of Agriculture of the Czech Republic under Projects No. MZE RO 0418 and No. QJ1510163. The authors thank Prof. Y. Xi for providing them with experimental materials and information.

## References

- Akkaya M.S., Buyukunal-Bal E.B. (2004): Assessment of genetic variation of bread wheat varieties using microsatellite markers. *Euphytica*, 135: 179–185.
- Araus J.L., Ferrio J.P., Buxó R., Voltas J. (2007): The historical perspective of dryland agriculture: lessons learned from 10 000 years of wheat cultivation. *Journal of Experimental Botany*, 58: 131–145.
- Bradová J., Matějová E. (2008): Comparison of the results of SDS PAGE and chip electrophoresis of wheat storage proteins. *Chromatographia*, 67: 83–88.
- Branlard G., Dardevet M., Saccomano R., Lagoutte F., Gourdon J. (2001): Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica*, 119: 59–67.
- Cattivelli L., Rizza F., Badeck F.W., Mazzucotelli E., Mastrolongo A.M., Francia E., Marè C., Tondelli A., Stanca A.M. (2008): Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Research*, 105: 1–14.
- Condon A.G., Richards R.A., Farquhar G.D. (1993): Relationships between carbon isotope discrimination and water-use efficiency for dryland wheat. *Australian Journal of Agricultural Research*, 44: 1693–1711.
- Ehdaie B., Hall A.E., Farquhar G.D., Nguyen H.T., Waines J.G. (1991): Water-use efficiency and carbon isotope discrimination in wheat. *Crop Science*, 31: 1282–1288.
- Farquhar G.D., Richards R.A. (1984): Isotopic composition of plant carbon correlates with water use efficiency of wheat. *Australian Journal of Plant Physiology*, 11: 539–552.
- He Z.H., Rajaram S., Xin Z.Y., Huang G.Z. (eds.) (2001): *A History of Wheat Breeding in China*. Mexico, CIMMYT.
- Payne P.I., Lawrence G.J. (1983): Catalogue of alleles or the complex loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which coded for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Research Communications*, 11: 29–35.
- Perrier X., Jacquemoud-Collet J.P. (2006): DARwin Software. Available at <http://darwin.cirad.fr/darwin> (accessed Oct 2, 2017).
- Plaschke J., Ganai M.W., Röder M. (1995): Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theoretical and Applied Genetics*, 91: 1001–1007.
- Prášil I., Rogalewicz V. (1989): Accuracy of wheat winter-hardiness evaluation by a provocation method in natural conditions. *Genetika a Šlechtění (Praha)*, 25: 223–230.
- Rebetzke G.J., Condon A.G., Richards R.A., Farquhar G.D. (2002): Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Science*, 42: 739–745.
- Röder S.M., Korzun V., Wendehake K., Plaschke J., Tixier M.H., Leroy P., Ganai M.W. (1998): A microsatellite map of wheat. *Genetics*, 149: 2007–2023.
- Roussel V., Leišová L., Exbrayat F., Stehno Z., Balfourier F. (2005): SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. *Theoretical and Applied Genetics*, 111: 162–170.
- Saghai-Marouf M.A., Soliman K.M., Jorgensen R.A., Allard R.W. (1984): Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences USA*, 81: 8014–8018.
- Song Q.J., Shi J.R., Singh S., Fickus E.W., Costa J.M., Lewis J., Gill B.S., Ward R., Cregan P.B. (2005): Development and mapping of microsatellite (SSR) markers in wheat. *Theoretical and Applied Genetics*, 110: 550–560.
- Zheleva D., Todorovska E., Christov N., Ivanov P., Ivanova I., Todorov I. (2007): Assessing the genetic variation of Bulgarian bread wheat varieties by biochemical and molecular markers. *Biotechnology and Biotechnological Equipment*, 21: 311–321.

Received for publication December 11, 2017

Accepted after corrections May 4, 2018

Published online June 13, 2018