GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic diversity and relationships among accessions of five crested wheatgrass species (Poaceae: *Agropyron*) based on gliadin analysis

S.Y. Chen, X. Ma, X.Q. Zhang, L.K. Huang and J.N. Zhou

Key Laboratory of Grassland Science, Sichuan Province,
Department of Grassland Science, Animal Science and Technology College,
Sichuan Agricultural University, Yaan, Sichuan, China

Corresponding author: X.Q. Zhang
E-mail: zhangxq@sicau.edu.cn

Genet. Mol. Res. 12 (4): 5704-5713 (2013)
Received December 6, 2012
Accepted March 18, 2012
Published November 18, 2013
DOI http://dx.doi.org/10.4238/2013.November.18.19

ABSTRACT. *Agropyron* Gaertn. is the most important genus in Triticeae (Poaceae), which includes many forage grasses with high economic value. The genetic diversity and relationships of 36 accessions from five crested wheatgrass species were analyzed by gliadin markers. A total of 54 product bands were detected after acid polyacrylamide gel electrophoresis (A-PAGE), of which 100% were polymorphic. The genetic similarity coefficient based on Nei-Li's method ranged from 0.065 to 0.755 with an average of 0.451. The Shannon diversity information index showed that there was a high level of genetic diversity among the accessions. An unweighted pair group method with arithmetic average (UPGMA) dendrogram was constructed based on the Nei-Li's genetic similarity coefficients, which showed the phylogenetic relationships among accessions of different species. Analysis of molecular variance (AMOVA) showed that the proportion of variance explained by inter- and intraspecific variance was 9.34 and 90.66%, respectively, which revealed that the genetic variations within species were higher than the variations among species. Based

on pairwise genetic distances (Φ_{ST}) among species, the cluster analysis indicated that *A. mongolicum* had a low-affinity relationship with other species, while *A. fragile* showed a close relationship with *A. cristatum* ssp *pectinatum*. Finally, the implications of the results for the taxonomy of *Agropyron* were discussed.

Key words: *Agropyron* Gaertn.; Genetic diversity; Gliadin; Phylogenetic relationship

INTRODUCTION

Agropyron Gaertn., one of the most important perennial genera in Triticeae (Poaceae), encompasses 10-15 species, which are commonly called the crested wheatgrass complex, and is composed of a series of diploid, tetraploid, and hexaploid species containing the basic P genome (Dewey, 1984; Asay and Jensen, 1996). *Agropyron* species are native to Europe and Asia, especially in the low temperature Altiplano and Sandlot regions in Eurasia, and some species have also been introduced and widely cultivated in North America (Johnson, 1986; Clayton et al., 2006). Most *Agropyron* species are excellent sources of forage and habitat for livestock and wildlife, and they are also valued for weed control, habitat use, soil stabilization, and watershed management (Wang, 2011). In addition, some *Agropyron* species possess many excellent genes that make them resistant to abiotic stress and major diseases; these genes can be transferred to cultivated cereal crops, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.), to develop new breeding materials, and they play an important role in the genetic improvement of cereal crops (Sharma and Gill, 1983; Dong et al., 1992; Wu et al., 2006).

The taxonomy of *Agropyron* underwent a major change and has been the object of considerable controversy. *Agropyron* used to be the largest genus in Triticeae, consisting of approximately 100 species that occur worldwide and have the characteristic of a single spikelet per node (Sakamoto, 1964). More recently, new taxonomic revisions have been proposed that are based on genomic or biological relationships as well as plant morphology. These revisions proposed that *Agropyron* should be restricted to species of the crested wheatgrass complex, a polyploid series based on the P genome (Dewey, 1984; Yen et al., 2005).

Traditionally, the delimitation of the crested wheatgrass complex mainly depended on the spike morphology, which varied in a continuous fashion from broad, pubescent, pectinate spikes to narrow, linear, glabrous spikes (Dewey and Asay, 1982). In the complex, *A. cristatum* (L.) Gaertn. and *A. fragile* (Roth) P. Candargy are the most used and widely distributed species, and they are commercially important. *A. cristatum* has short broad spikes that taper at the top, small seeds, and short stature; compared to *A. cristatum*, *A. fragile* has finer leaves and stems and narrower and awnless glumes and lemmas, and the spikelets are more ascending, giving the spike a narrow, oblong, subcylindrical shape (Clayton et al., 2006). Similarly, *A. imbricatum* (MB) Roem et Schult and *A. pectinatum* Roem et Schult are morphologically very similar to each other and to *A. cristatum* (L.) Gaertn.; they are differentiated by the level of pubescence and the spacing between spikelets. Tzvelev (1976) reclassified them as subspecies or varieties of *A. cristatum*, namely *A. cristatum* ssp *imbricatum* (Roem. & Schult.) Beck and *A. cristatum* ssp *pectinatum* (M. Bieb.). The cytology data indicated that these three species

differed in the absence or presence of B chromosomes and the position of satellites (Asghari et al., 2007). Moreover, *A. mongolicum* Keng, which is indigenous to China, is distinguished from *A. cristatum* by its narrow linear spikes. It was also postulated that *A. fragile* was an autopolyploid of *A. mongolicum* (Asay et al., 1992). However, the taxonomy is still controversial and difficult because the morphological characteristics that are used to distinguish its taxa are at least partially under environmental control and may not reflect their genetic basis (Sun and Li, 2006). Furthermore, the complex is known for its morphological variability, but little is known about the genetic basis and systematic relationships, especially at the biochemical and molecular levels.

Gliadins, the important proportions of storage protein in the endosperm of Triticeae species, are encoded by highly conserved multigenic families, and differences in copy number result in gliadin polymorphisms (Shewry et al., 1999). The great variability of these proteins, which is a consequence of their neutral nature at the evolutionary level, can substantially contribute to the analysis of evolutionary forces that cause genetic variation and differentiation (Alvarez et al., 2006). Despite having fewer detectable loci than DNA molecular markers, gliadin markers still have great application potential in the identification of genotypes and characterization of genetic relationships between plant germplasms because of their simplicity, speed, and high repeatability (Ma et al., 2012). Hitherto, gliadin has been used as a powerful tool in *Apropyron* and other Triticeae species to reveal genetic diversity and population differentiation and to elucidate phylogenetic relationships and taxonomic problems (Che and Li, 2007; Özbek et al., 2011). The objectives of this study were therefore to detect genetic diversity and examine inter- or intraspecific systematic relationships among the *Agropyron* accessions.

MATERIAL AND METHODS

Plant materials

A total of 36 *Agropyron* accessions belonging to *A. mongolicum*, *A. cristatum*, *A. cristatum* ssp *imbricatum*, *A. cristatum* ssp *pectinatum*, and *A. fragile* were used in this study (Table 1). All of the seeds were obtained from the United States Department of Agriculture - Agricultural Research Service (USDA-ARS), Regional Plant Introduction Station, Pullman, Washington, USA.

Gliadin extraction and electrophoresis

Twenty seeds of each accession were randomly selected and crushed into fine powders for the gliadin extraction. The gliadin was extracted with a solution of 25% 2-chlorohydrin (v/v) and 0.05% methyl green (w/v), and it was fractionated by standard acid polyacrylamide gel electrophoresis (A-PAGE) according to the procedure of Draper (1987). The gel concentration was 10% (w/v), and the cross linker was 3.3% (w/w). Electrophoresis was carried out at 500 V for 3 h at a constant temperature of 15°C in pH 3.1 electrophoresis buffer. After electrophoresis, the gel was fixed in 10% trichloroacetic acid (TCA) (w/v) and stained with 1% Coomassie brilliant blue R-250 (w/v). Destaining was carried out with 7% acetic acid and tap water (v/v). Moreover, the Canadian wheat cultivar Marquis (*Triticum aestivum* 'Marquis') was taken as the standard for recording the band patterns.

Table 1. *Agropyron* species used in the study.

No.	Accessions No.	Species (Abbreviation)	Geographic origins
1	PI499393	*A. mongolicum* (*A. m*)	Nei Monggol, China
2	PI531543	*A. mongolicum* (*A. m*)	Nei Monggol, China
3	PI598482	*A. mongolicum* (*A. m*)	China
4	PI598460	*A. mongolicum* (*A. m*)	China
5	PI564856	*A. cristatum* (*A. c*)	Nei Monggol, China
6	PI499566	*A. cristatum* (*A. c*)	Xinjiang, China
7	PI499381	*A. cristatum* (*A. c*)	Nei Monggol, China
8	w613146	*A. cristatum* (*A. c*)	Xinjiang, China
9	PI401026	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Kars, Turkey
10	PI564882	*A. cristatum* var. *pectinatum* (*A. c. p*)	Kazakhstan
11	PI564884	*A. cristatum* var. *pectinatum* (*A. c. p*)	Kazakhstan
12	PI370649	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Russian Federation
13	PI370653	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Novosibirsk region, Russian Federation
14	PI370650	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Russian Federation
15	PI273734	*A. cristatum* var. *pectinatum* (*A. c. p*)	Voronezh, Russian Federation
16	PI494617	*A. cristatum* var. *pectinatum* (*A. c. p*)	Constanta, Romania
17	PI547323	*A. cristatum* var. *pectinatum* (*A. c. p*)	Russian Federation
18	PI326204	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Kustanai region, Kazakhstan
19	PI401027	*A. cristatum* subsp. *pectinatum* (*A. c. p*)	Sakarya, Turkey
20	PI401013	*A. cristatum* var. *imbricatum* (*A. c. i*)	Turkey
21	PI401012	*A. cristatum* var. *imbricatum* (*A. c. i*)	Orenburg region, Turkey
22	PI314605	*A. cristatum* var. *imbricatum* (*A. c. i*)	Alma Ata, Kazakhstan
23	PI229574	*A. cristatum* var. *imbricatum* (*A. c. i*)	Azerbaijan, Iran
24	PI401011	*A. cristatum* var. *imbricatum* (*A. c. i*)	Rostov region, Turkey
25	PI285205	*A. cristatum* var. *imbricatum* (*A. c. i*)	Russian Federation
26	PI401028	*A. fragile* (*A. f*)	Saratov region, Turkey
27	PI326206	*A. fragile* (*A. f*)	Aktyubinsk region, Kazakhstan
28	PI108434	*A. fragile* (*A. f*)	Kazakhstan
29	PI314606	*A. fragile* (*A. f*)	Alma Ata, Kazakhstan
30	PI440494	*A. fragile* (*A. f*)	Dzhambul, Kazakhstan
31	PI369522	*A. fragile* (*A. f*)	Siberia, Russian Federation
32	PI273736	*A. fragile* (*A. f*)	Aktyubinsk, Kazakhstan
33	PI370655	*A. fragile* (*A. f*)	Russian Federation
34	PI276710	*A. fragile* (*A. f*)	Moscow, Russian Federation
35	PI325184	*A. fragile* (*A. f*)	Stavropol, Russian Federation
36	PI315366	*A. fragile* (*A. f*)	Stavropol, Russian Federation

Data analysis

The gliadin bands were scored manually for their presence (1) or absence (0) to construct the data matrix. The Nei-Li's genetic similarity (GS) coefficients were calculated, which were also used to compute principal coordinate analysis (PCoA) and construct the dendrogram using unweighted pair group method with arithmetic average (UPGMA) cluster analysis in the NTSYS 2.1 software (Gower, 1966; Nei and Li, 1979; Rohlf, 2000). The Shannon index was also applied to estimate the genetic diversity among the accessions as $H = -\Sigma\pi i \ln\pi i$, where πi is the frequency of a band in the group or subgroup. Additionally, to study the partition of inter- and intraspecific genetic variations of the gliadin, the analysis of molecular variance (AMOVA) was also conducted with the program WIN AMOVA 1.55 (Excoffier et al., 1992). The interspecific genetic distances were analyzed by the phi statistic (Φ_{ST}). The number of permutations was set at 1000 for AMOVA and the significance test of the interspecific genetic distances.

RESULTS

A-PAGE profile

Up to 54 gliadin bands were detected among the 36 accessions of five crested wheat-

grass species, of which 100% were polymorphic in the examined accessions. Forty-three (37 polymorphic bands), 44 (40 polymorphic bands), 51 (51 polymorphic bands), 49 (48 polymorphic bands), and 52 (52 polymorphic bands) bands were detected from *A. mongolicum*, *A. cristatum*, *A. cristatum* ssp *pectinatum*, *A. cristatum* ssp *imbricatum*, and *A. fragile*, respectively (Table 2). The largest number of bands was observed in *A. cristatum* ssp *pectinatum*, while the least was observed in *A. mongolicum*. The gliadin markers in the study could separate all of the accessions, and each accession had a unique band pattern.

Table 2. Detected bands and Shannon index of five *Agropyron* species.

Species	Number of total bands	Polymorphic bands and rate	Shannon index (*H*)
A. mongolicum	43	37 (86.05%)	0.3904
A. cristatum	44	40 (90.91%)	0.4051
A. cristatum ssp *imbricatum*	49	48 (98.96%)	0.4453
A. cristatum ssp *pectinatum*	51	51 (100%)	0.4613
A. fragile	52	52 (100%)	0.4639
Total	56	56 (100%)	0.5240

GS coefficient and variability

The GS coefficient among accessions based on Nei-Li's method ranged from 0.065 (PI531543, *A. mongolicum* and PI564856, *A. cristatum*) to 0.755 (PI370650, *A. cristatum* ssp *pectinatum* and PI285205, *A. cristatum* ssp *imbricatum*) with an average of 0.451. At the intraspecific level, different accessions of each species revealed high similarity, and the GS value varied between 0.411 and 0.469. The highest similarity value was 0.469 between *A. cristatum* ssp *pectinatum* and *A. fragile*, while the lowest value was 0.411 between *A. mongolicum* and *A. cristatum* ssp *imbricatum*. The Shannon diversity information index of all accessions was 0.5240, and indices of *A. mongolicum*, *A. cristatum*, *A. cristatum* ssp *imbricatum*, *A. cristatum* ssp *pectinatum*, and *A. fragile* were 0.3904, 0.4051, 0.4453, 0.4613, and 0.4639, respectively (Table 2). This finding showed that there was a high level of genetic diversity among the accessions, and the genetic variations within the species were higher than the variations among the species of the complex.

Cluster and PCoA analysis

The dendrogram constructed from Nei-Li's genetic similarity coefficient with UPGMA analysis revealed several main clusters (Figure 1), which revealed the genetic relationships among the *Agropyron* species at the inter- and intraspecific levels. Cluster I contains one accession of *A. cristatum* from China and one accession of *A. cristatum* ssp *imbricatum* from Turkey. In cluster II, there was only one accession of *A. cristatum* ssp *imbricatum* from Iran. Cluster III contained two accessions from the same region of the Russian Federation, *A. cristatum* ssp *pectinatum* and *A. fragile*. Finally, cluster IV included the remainder of the accessions, which could then be further divided into five subgroups. Subgroup IV-1 consisted of three *A. mongolicum* accessions from China. Subgroup IV-2 contained nine accessions belonging to the five species. Subgroup IV-3 included 13 accessions from the four species, except *A. mongolicum*. Subgroup IV-4 was composed of three *A. fragile* accessions and one *A. cristatum* ssp *pectinatum* accession, all of which were from Kazakhstan. Finally, two other *A. fragile* accessions from the Russian Federation were clustered in subgroup IV-5.

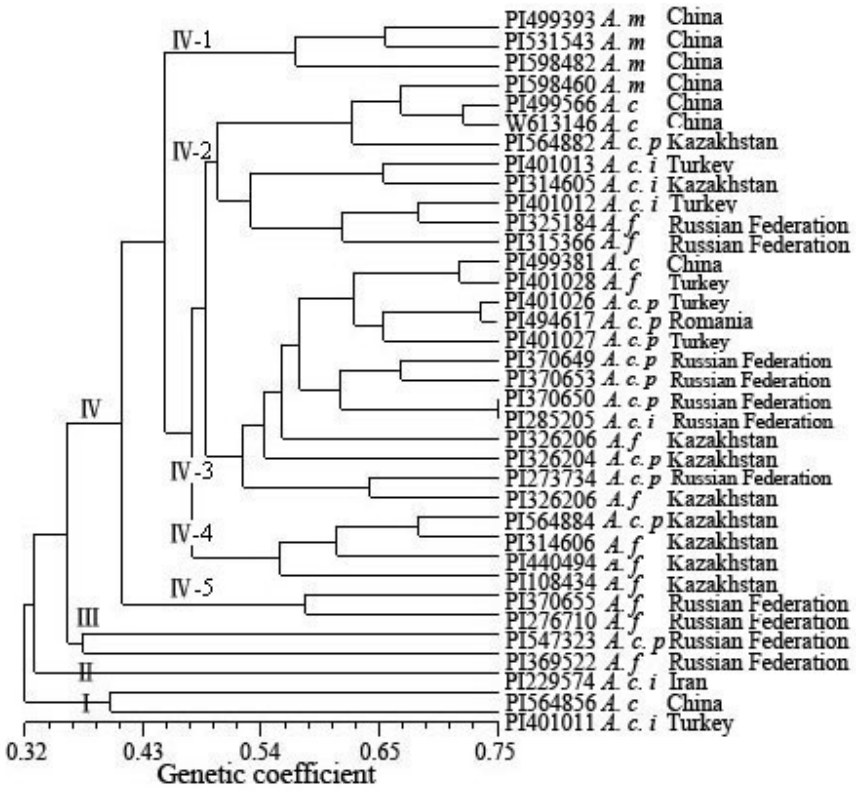

Figure 1. UPGMA dendrogram generated by Nei-Li's genetic similarity coefficients based on gliadin analysis.

PCoA was performed based on the genetic similarity matrix to better understand relationships among the accessions of different species (Figure 2). The three most informative principal coordinate components accounted for 56.76% of the variations, of which Principal Coordinate 1, 2, and 3 explained 47.37, 5.01, and 4.38% of the variation, respectively. The PCoA also revealed groupings that were similar to those from the UPGMA cluster dendrogram. The PCoA results indicated that there was a clear separation between *A. mongolicum* and other *Agropyron* species (Figure 2).

AMOVA analysis

AMOVA analysis revealed that molecular variation accounted for 9.34 and 90.66% of the variation among and within species, respectively. Then, pairwise Φ_{ST} values (Φ_{ST} distances) were obtained based on the AMOVA analysis, which indicated the genetic distances among the species. Table 3 showed that the distance between *A. cristatum* ssp *pectinatum* and *A. fragile* was the lowest (0.0250), and the distance between *A. mongolicum* and *A. cristatum* ssp *imbricatum* was the highest (0.2106). The UPGMA dendrogram (Figure 3) based on pairwise Φ_{ST} distances clearly showed the relationships among the five species, which were congruent with the previous cluster analysis of 36 accessions based on Nei-Li's GS coefficients.

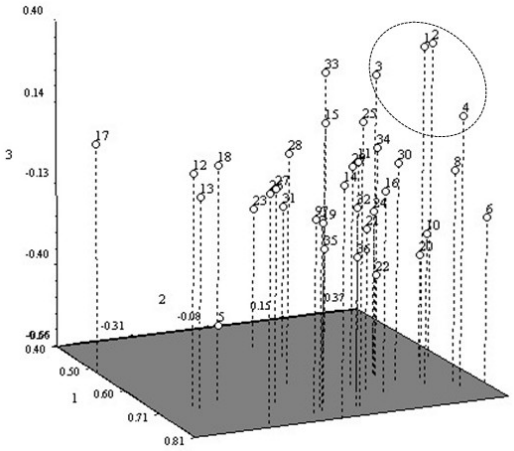

Figure 2. Relationships among the accessions of five *Agropyron* species visualized by principal coordinate analysis (PCoA) of gliadin-based genetic similarities.

Table 3. Pairwise Φ_{ST} distance of five *Agropyron* species.

Species	*A. mongolicum*	*A. cristatum*	*A. cristatum* ssp *imbricatum*	*A. cristatum* ssp *pectinatum*	*A. fragile*
A. mongolicum	0.0000				
A. cristatum	0.1859	0.0000			
A. cristatum ssp *imbricatum*	0.2106	0.1390	0.0000		
A. cristatum ssp *pectinatum*	0.1611	0.0400	0.0619	0.0000	
A. fragile	0.1537	0.0817	0.0425	0.0250	0.0000

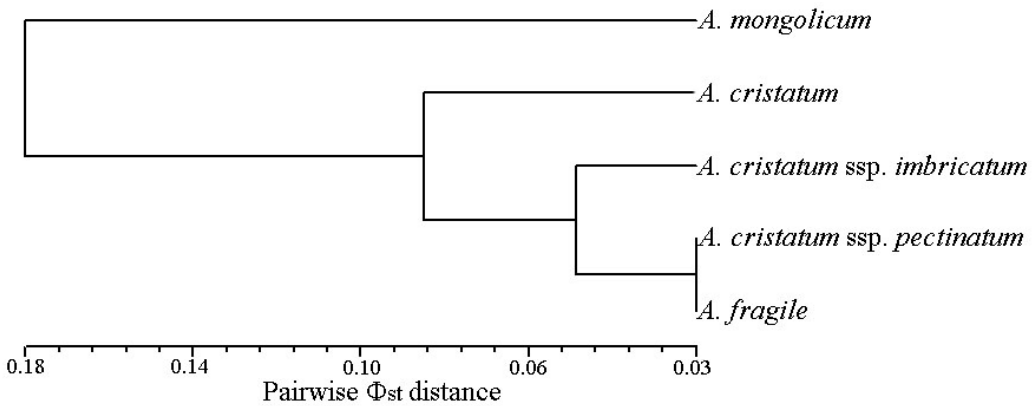

Figure 3. UPGMA clustering of five Agropyron species based on pairwise Φ_{ST} distances.

DISCUSSION

In the present study, substantial genetic diversity was revealed among accessions of different species, and the gliadin marker was also proved as a powerful tool in cultivar or acces-

sion identification. Che and Li (2007) stated that a total of 81 gliadin bands were detected from 40 populations of *A. mongolicum* that was indigenous to Northern China, which showed that populations from similar eco-geographical situations could be clustered together. Furthermore, gliadin was widely applied in two other important wheatgrass species, *Elymus nutans* and *E. sibiricus*, which revealed considerable genetic variation and distinct geographical divergence among the accessions (Miao et al., 2011; Ma et al., 2012). Therefore, they also indicated that gliadin was a rapid, simple, and efficient marker system for genetic research of Triticeae species.

The results of the cluster analysis showed that most of the different accessions in a single species and the species from the same area were clustered together. Therefore, the cluster analysis based on gliadin was closely related to the geographical origins. However, a few accessions (for instance, cluster II) could not be clustered in any group and presented specificities, which might have resulted from various selection forces that tended to produce genetic heterogeneity in different small niches caused by different ecological factors. Nevo et al. (1997) analyzed the protein variation in natural populations both at macro- and micro-geographical scales and concluded that the patterns of protein diversity were often related to ecological factors. Unfortunately, we did not detect any clear separation between different species or subspecies, which indicated that they had similar genetic bases and a great deal of genetic diversity among species.

In this study, *A. mongolicum* and *A. cristatum* had relatively low diversity index values, while *A. fragile* and *A. cristatum* ssp *pectinatum* had relatively high values. This finding might be associated with the geographical origins of the accessions and their complicated habitats because the accessions of the 1st two species were mainly from China, and the last 2 species in this study were from a wide geographical area. The role of ecological factors has been emphasized in determining the extent and distribution of genetic diversity in crop wild relatives and Triticeae species (Nevo, 1998; Chen et al., 2009). Moreover, significant correlation was also found between genetic diversity and sample size; therefore, this result might also be related to the sample size (Godt et al., 1996). Additionally, the results of AMOVA and Shannon index both revealed that the genetic variations within the species were higher than the variations among the species of the complex. Mellish et al. (2002) also reported that the majority of the AFLP variance (88%) was found within the *Agropyron* species population, which may be because they are all outcrossing species.

Hsiao et al. (1986) described that although *A. mongolicum* and *A. cristatum* had similar genome lengths, there were some structural rearrangements between their P genomes, which were called the P_c and P_m genome. Similarly, *A. mongolicum* was not closely related to *A. cristatum* and other species in the study. The pairwise Φ_{ST} distances indicated that *A. mongolicum* showed a relatively low affinity relationship with other species, which was also similar to the results of a previous study that was based on amplified fragment length polymorphism (AFLP) markers (Mellish et al., 2002). Nevertheless, *A. cristatum* ssp *pectinatum* showed a close relationship with *A. fragile* and not with *A. cristatum* or with *A. cristatum* ssp *imbricatum*, which could have resulted because of the overlap in the geographical distribution of the two species and because they possess similar habitats.

In conclusion, this study indicated that gliadin was a rapid, simple, and efficient marker for genetic analysis of *Agropyron* species. Then, on the basis of the gliadin analysis, substantial genetic diversity was detected among accessions of different species. It also showed a clear division between *A. mongolicum* and the 4 other species and a certain degree

of separation between *A. cristatum* and *A. fragile*. Nonetheless, it seemed that these results did not support the classification of *A. cristatum* ssp *imbricatum* and *A. cristatum* ssp *pectinatum* as subspecies of *A. cristatum*. Therefore, in order to draw convincing conclusions about the systematic relationships among the *Agropyron* species, further research needs to be conducted with more accessions from each species and additional analysis methods.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Funds of China (#31072077 and #31101763) and the earmarked fund for Modern Agro-Industry Technology Research System (#CARS-35-05). The authors also thank the USDA for kindly supplying the seeds for the study.

REFERENCES

Alvarez JB, Broccoli A and Martin LM (2006). Variability and genetic diversity for gliadins in natural populations of *Hordeum chilense* Roem. et Schult. *Genet. Resour. Crop Evol.* 53: 1419-1425.

Asay KH and Jensen KB (1996). Wheatgrasses. In: Cool-Season Forage Grasses (Moser LE, Buxton DR and Casler MD, eds.). ASA-CSSA-SSSA, Madison, 691-724.

Asay KH, Jensen KB, Hsiao C and Dewey DR (1992). Probable origin of standard crested wheatgrass, *Agropyron desertorum* Fisch ex Link, Schultes. *Can. J. Plant Sci.* 72: 763-772.

Asghari A, Agayev Y and Fathi SA (2007). Karyological study of four species of wheat grass (*Agropyron* sp.). *Pak. J. Biol. Sci.* 10: 1093-1097.

Che YH and Li LH (2007). Genetic diversity of prolamines in *Agropyron mongolicum* Keng indigenous to northern China. *Genet. Resour. Crop Evol.* 54: 1145-1151.

Chen SY, Ma X, Zhang XQ and Chen ZH (2009). Genetic variation and geographical divergence in *Elymus nutans* (Poaceae: Triticeae) from west China. *Biochem. Syst. Ecol.* 37: 722.

Clayton WD, Vorontsova MS, Harman KT and Williamson H (2006). GrassBase - The Online World Grass Flora. Available at [http://www.kew.org/data/grasses-db.html]. Accessed November 08, 2006.

Dewey DR (1984). The Genomic System of Classification as a Guide to Intergeneric Hybridization with the Perennial Triticeae. In: Gene Manipulation in Plant Improvement (Gustafson JP, ed.). Plenum Publishing Corp, New York, 209-279.

Dewey DR and Asay KH (1982). Cytogenetic and taxonomic relationships among three diploid crested wheatgrasses. *Crop Sci.* 22: 645-650.

Dong YS, Zhou RH, Xu SJ, Cauderon Y, et al. (1992). Desirable characteristics in perennial Triticeae collected in China for wheat improvement. *Hereditas* 116: 175-178.

Draper SR (1987). ISTA Variety Committee - Report of the working group for biochemical tests for cultivar identification 1983-1986. *Seed Sci. Tech.* 15: 431-434.

Excoffier L, Smouse PE and Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Godt MJW, Johnson BR and Hamrick JL (1996). Genetic diversity and population size in four rare southern appalachian plant species. *Conserv. Biol.* 10: 796-805.

Gower JC (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 325-338.

Hsiao C, Wang RRC and Dewey DR (1986). Karyotype analysis and genome relationships of 22 diploid species in the tribe Triticeae. *Can. J. Genet. Cytol.* 28: 109-120.

Johnson DA (1986). Seed and Seedling Relations of Crested Wheatgrass: A Review. In: Crested Wheatgrass: Its Values, Problems and Myths (Johnson KL, ed.). Proceedings of Symposium, Utah State University, Logan, 65-90.

Ma X, Chen SY, Zhang XQ, Bai SQ, et al. (2012). Assessment of worldwide genetic diversity of Siberian wild rye (*Elymus sibiricus* L.) germplasm based on gliadin analysis. *Molecules* 17: 4424-4434.

Mellish A, Coulman B and Ferdinandez Y (2002). Genetic relationships among selected crested wheatgrass cultivars and species determined on the basis of AFLP markers. *Crop Sci.* 42: 1662-1668.

Miao JM, Zhang XQ, Chen SY, Ma X, et al. (2011). Gliadin analysis of *Elymus nutans* Griseb. from the Qinghai-Tibetan Plateau and Xinjiang, China. *Grassland Sci.* 57: 127-134.

Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.

Nevo E (1998). Genetic diversity in wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. *Genet. Resour. Crop Evol.* 45: 355-370.

Nevo E, Apelbaum-Elkaher I, Garty J and Beiles A (1997). Natural selection causes microscale allozyme diversity in wild barley and a lichen at 'Evolution Caryon' Mt. Carmel, Israel. *Heredity* 78: 373-382.

Özbek Ö, Taşkın BG, Şan SK, Eser V, et al. (2011). Gliadin polymorphism in Turkish cultivated emmer wheat [*Triticum turgidum* L. ssp. *dicoccon* (Schrank) Thell.] landraces. *Plant Syst. Evol.* 296: 121-135.

Rohlf FJ (2000). NTSYSpc: Numerical Taxonomy and Multivariate Analysis System, Version 2.1 Exeter Software. Applied Biostatics Inc., New York.

Sakamoto S (1964). Cytogenetical problems in *Agropyron* hybrids. *Seiken Jiho* 16: 38-47.

Sharma HC and Gill BS (1983). Current status of wide hybridization in wheat. *Euphytica* 32: 17-31.

Shewry PR, Tatham AS and Halford NG (1999). The Prolamins of the Triticeae. In: Seed Proteins (Shewry PR and Casey R, eds.). Kluwer Academic Publishers, Dordrecht, 35-78.

Sun GL and Li W (2006). Molecular diversity of species of the *Elymus trachycaulus* complex and their relationships to non-North American taxa. *Plant Syst. Evol.* 256: 179-191.

Tzvelev NN (1976). Tribe 3. Triticeae Dum. In: Poaceae URSS Nauka Publishing House, St. Leningrad, 105-206.

Wang RRC (2011). *Agropyron* and *Psathyrostachys*. In: Wild Crop Relatives: Genomic and Breeding Resources (Kole C, ed.). Springer-Verlag, Berlin Heidelberg, 77-83.

Wu J, Yang X, Wang H, Li H, et al. (2006). The introgression of chromosome 6P specifying for increased numbers of florets and kernels from *Agropyron cristatum* into wheat. *Theor. Appl. Genet.* 114: 13-20.

Yen C, Yang JL and Yen Y (2005). Hitoshi Kihara, Áskell Löve and the modern genetic concept of the genera in the tribe Triticeae (Poaceae). *Acta Phytotaxon. Sin.* 43: 82-93.