Genetic variation of wheat landraces in Afghanistan

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ABSTRACT

To characterize wheat landraces collected from Afghanistan, intra- and inter-regional genetic variation were analyzed. The landraces we investigated had been collected during 1955~ 78 and maintained by genebank in Japan, which should be valuable genetic resources. Two experiments were conducted in this study, namely, storage protein variation analysis, and genomic AFLP analysis. Results indicated that the genebank collections of Afghan wheat are true landraces without contamination of modern varieties or introduction of alien genetic factors. The results also indicated that the genetic diversity of Afghan wheat is generally high between regions, but not so high within regions, suggesting a bygone occurrence of genetic bottleneck in the border area between Iran and Afghanistan. Both the landraces and the information obtained in this study should be useful as genetic resources for wheat breeding and available for reconstruction of Afghan agriculture and society.

INTRODUCTION

Afghanistan is one of the centres of genetic diversity for many important crops. However, long years of war and drought in the last several decades have lost the region's genetic resources and destroyed the base of agriculture. Afghan wheat landraces were collected in three botanical expeditions organized by the Kyoto University Scientific Expedition to Karakoram and Hindukush (we called it "KUSE 1955"), Thomas's expedition ("Thomas 1965"), and Kyoto University Scientific Expedition to Southwestern Eurasia ("SGK 1978"; Kihara *et al*, 1965; Kawahara *et al*, 1987; Kawahara 2005). These materials have been maintained in Plant Germplasm Institute, Kyoto University.

In this study we used these genetic resources to evaluate and characterize wheat landraces from Afghanistan. Moreover, we investigated the genetic relationship between landraces of Afghanistan and neighbouring countries.

Here we analysed genetic diversity of high molecular glutenin subunits (HMW-GS) which is the major endosperm storage proteins in wheat and amplified fragment length polymorphism (AFLP) using the genebank collection. Genotype of HMW-GS much affects the bread making quality, therefore diversity analysis of HMW-GS provide an opportunity find novel valuable genetic resources. It also gives us useful information to understand the process of spread of bread wheat from western to eastern Asia under agricultural selection. On the other hand, AFLP analysis demonstrates the neutral molecular variation for whole genome.

In this study, the neutral DNA marker, genetic characteristics of Afghan wheat landraces and the way of spread of bread wheat in central Asia are discussed.

MATERIALS AND METHODS

Two sets of Afghan landraces were used in this study. The first consisted of 475 accessions of hexaploid wheat originated from Afghanistan (410), Iran (33) and Pakistan (32), which were used for HMW-GS analysis. These accessions were divided into eleven groups according to collection sits. The second set is 91 accessions from Afghanistan (30), Iran (31) and Pakistan (30) which were only KUSE 1955 collection. This second was set for used for AFLP. All the materials are the collection of Plant Germplasm Institute, Kyoto University, provided throughout National BioResources Project KOMUGI.

Analysis of HMW-GS was carried out on single kernel of at least 3 to 4 seeds for each accession, in accordance with the SDS-PAGE methods of Payne *et al*. (1983).

AFLP analysis was conducted following the method of Vos *et al*. (1996). Five primer combinations were used in this study.

RESULTS

HMW-GS analysis

The allele frequency of *Glu-A1c* encoding null subunits was the highest 74.6% at the *Glu-A1* locus and followed *Glu-A1b* in Afghan landraces. The similar results were obtained in Iranian accessions (Table1), although in this case the highest proportion at *Glu-A1* allele was *Glu-A1b*, which shared 50.0% in Pakistan.

At the *Glu-B1* locus, *Glu-B1b* (encoding subunits 7+8) shared 72.7% in Afghan landraces. The frequencies were showed in Table 1. Most of Iranian and Pakistani ones carried *Glu-B1b* also. However, frequency of the next *Glu-B1* allele (see Table 1) was different among Afghan, Iranian and Pakistani landraces.

Table 1. Allelic frequency (%) of Glu-1 HMW –GS in wheat landraces from Afghanistan, Iran and Pakistan

Glu-A1	Region			*Glu-B1*	Region			*Glu-D1*	Region		
Allele	AFG	IRN	PAK	Allele	AFG	IRN	PAK	Allele	AFG	IRN	PAK
a	8.5	6.1	25.0	*b*	87.3	72.7	50.0	*a*	84.9	81.8	75.0
b	16.8	15.2	50.0	*c*	3.2	0.0	9.4	*c*	0.0	9.1	9.4
c	74.6	78.8	25.0	*d*	2.4	0.0	0.0	*d*	2.7	0.0	0.0
				e	0.7	12.1	3.1	*h*	0.7	0.0	9.4
				i	1.2	15.2	3.1	*l*	1.5	6.1	0.0
				f	0.0	0.0	28.1	*m*	6.1	0.0	0.0
				aj	3.7	0.0	3.1	*br(s)*	0.2	0.0	0.0

Country code indicates the following: AFG, Afghanistan IRN, Iran; PAK, Pakistan

We identified a new subunit which tentatively named 2.8+12 encoded by a new allele *Glu-D1br(s)* in Afghan landrace. Subunit 2.8 showed slightly higher molecular weight than subunit 7. At the *Glu-D1* locus, the major allele was *Glu-D1a* (2+12) with a frequency of 84.9% in Afghan wheat landraces. Most of the Iranian landraces and the Pakistani landraces also had *Glu-D1a*. In Iranian landraces *Glu-D1c* and *Glu-D1l* followed in allele frequencies. On the other hand, in Pakistani landraces, *Glu-D1c* and *Glu-D1h* followed.

Genetic diversity of HMW-GS

Genetic diversity of HMW-GS for each regional group is shown in Table 2. Within Afghanistan, it was shown that PIC value increased from the west (0.192) to the east regions (east 0.525, south east 0.506, and north east 0.606). On the contrary, genetic diversity tends to increase from the east (0.182) to west (0.838) region in Iran. The genetic diversity in Pakistan is very high in both central (0.798) and north (0.875) regions.

Table 2. Genetic diversity of region (HMW-GS)

Region	Total no. of pattern	PIC
AFG		
North east	17	0.606
South east	3	0.560
East	9	0.525
North	2	0.435
Central	17	0.384
West	4	0.192
IRN		
East	7	0.182
Central	4	0.328
West	2	0.838
PAK		
Central	10	0.798
North	8	0.875

* PIC= $1-\sum_{i=1}^{n} pi^2$ pi=frequency of Glu-1 pattern

Genetic diversity revealed by AFLP

In order to investigate total genetic variation, intra- and inter-region variability was has by Manhattan value. The genetic valuations of intraregional groups are shown in Table 3. The genetic variation of entire Afghan wheat landraces was 1.66, which was lower than the values of entire Iranian and Pakistani landraces (1.96 and 2.11, respectively). In Afghan landraces each regional group had the similar level of variation (1.59, 1.39, and 1.38). As for Iranian landraces, three regional groups showed different level of variation. Eastern Iran had the lowest variation (1.02) among all regions.

Table 3. Genetic diversity of each region from AFLP analysis

Region	Number of samples	Manhattan value[1)]
AFG		
North	9	1.59
South East	11	1.39
East	10	1.38
All	30	1.66
IRN		
East	10	1.02
Central	10	2.26
West	11	2.01
All	31	1.96
PAK		
North	15	1.93
Central	15	1.59
All	30	2.11

Manhattan value: $Dm(x,y) = \Sigma\,[k=1 \sim p](|x(k) - y(k)|) \times 10^{-1}$
k=Numbers of bands

Interregional genetic valuation is shown in Table 4. The highest level of variation was observed in the comparison between Central Iran and North Pakistan (2.39). South Eastern Afghanistan was relatively close to Eastern Iran (1.44). The genetic distance between Iran east and all Afghan regions (1.44, 1.45 and 1.66) are closer than all the other comparison. The genetic distances among the three Afghan regions were also small.

Table 4. Genetic distance between regions (Manhattan value×10^{-1})

Region	IRN East	IRN Central	IRN West	AFG South East	AFG East	AFG North	PAK Central
IRN Central	1.80						
IRN West	1.80	2.29					
AFG South East	1.44	2.07	2.05				
AFG East	1.66	2.18	2.14	1.62			
AFG North	1.45	2.15	2.12	1.59	1.78		
PAK Central	1.72	2.10	2.15	1.68	1.72	1.80	
PAK North	2.05	2.39	2.34	2.11	2.14	2.14	2.11

DISCUSSION

After the formation of hexaploid bread wheat in southern coastal area of Caspian Sea, its variants had been spread to the east and the west. The Silk Road is considered to be one of the main ways for the distribution of wheat to eastern Asia. The Afghan area may have functioned as a distribution centre because of its geographical and cultural features. Since the landraces used in the present study were collected before modernization of Afghan agriculture, the materials had kept the original adaptive traits having wide variation.

The present study shows that the most of the Afghan wheat landraces had an identical genetic component of HMW-GS, which was common with Pakistani and Iranian landraces except for, *Glu-A1* locus for Pakistani landraces. The most frequent type of HMW-GS in this area was *Glu-A1c, Glu-B1b* and *Glu-C1a*. This is much different from the genotype of worldwide recent modern cultivars. Including the finding of a new allele, we can consider that Afghan wheat collection used in study possess the potential of genetic resource as traditional landraces.

Further we compared genetic diversity of HMW-GS and AFLP in Afghan, Iranian and Pakistani regions. The two data sets showed similar tendency. The results show genetic diversity was low in border areas between Iran and Afghanistan. Although the levels of genetic variation are different, the genetic composition was not very different among the regions in Afghanistan and Iran. Interestingly, the Pakistani wheat landraces had unique genetic components and higher levels of variation compared to Afghan and Iranian landraces. These results indicate that there must be a genetic bottleneck around the border between Afghanistan and Iran, which was probably caused by an agricultural selective pressure or decreasing of population size during spread. Pakistan may have been the second diversity centre during the spread of hexaploid wheat.

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