

## Exploration of genetic diversity among Xinjiang *Triticum* and *Triticum polonicum* by AFLP markers

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**Abstract.** Seventy-two Xinjiang *Triticum* and *Triticum polonicum* accessions were subjected to AFLP analyses to discuss the origin of *Triticum petropavlovskyi*. A total of 91 putative loci were produced by four primer combinations. Among them 56 loci were polymorphic, which is equivalent to 61.53 % of the total number of putative loci. Genetic diversity among 11 *T. petropavlovskyi* accessions was narrow due to the lowest number (32) of polymorphic loci among the wheat species. Forty four polymorphic loci were found in *T. aestivum* and *T. compactum*, whereas the highest polymorphism was observed in *T. polonicum*. On the basis of the UPGMA clustering and PCO grouping and genetic similarity estimates from the AFLPs, we noted that *T. petropavlovskyi* was more closely related to the Chinese accessions of *T. polonicum* than to *T. polonicum* from other countries. Two accessions of *T. aestivum* were grouped with *T. petropavlovskyi* in the UPGMA clustering. Both of them were similar to *T. petropavlovskyi* in respect of spike structure, i.e. the presence of awn, glume awn and also the presence of leaf pubescence. Six loci, which were commonly absent in Chinese *T. polonicum*, were also absent in almost all of the *T. petropavlovskyi* accessions. Findings of this study reduced the probability of an independent allopolyploidization event in the origin of *T. petropavlovskyi* and indicated a greater degree of gene flow between *T. aestivum* and *T. polonicum* leading to *T. petropavlovskyi*. It is most likely that the *P*-gene of *T. petropavlovskyi* hexaploid wheat was introduced from *T. polonicum* to *T. aestivum* via a spontaneous introgression or breeding effort.

**Keywords:** AFLP, diversity, Xinjiang *Triticum*, *Triticum aestivum*, *Triticum petropavlovskyi*, *Triticum polonicum*.

### Introduction

China has been proposed as a centre of diversity for wheat and *Triticum* species are abundant in the Xinjiang region (Yen et al. 1988). Chinese wheat landrace, Xinjiang rice wheat, *T. petropavlovskyi* Udacz. et Migusch., known as ‘Daosuimai’ or rice-head wheat, was found in the agricultural areas in the west part of the Talimu basin, Xinjiang, China in 1948. Xinjiang rice wheat is characterized by a long glume, similar to that of *T. turgidum* ssp. *polonicum*. It was brought to Russia by A.M. Gorski, and it was thought that Xinjiang rice wheat was a mutated form of *T. polonicum* (Jakubtsiner 1959). However,

its chromosome number is  $2n = 42$  and it was named as *T. petropavlovskyi* Udacz. et Migusch. (Udaczin and Miguschova 1970).

The origin of *T. petropavlovskyi* has been discussed based on the following hypotheses; (1) introgression from *T. polonicum* to *T. aestivum*; (2) independent allopolyploidization between tetraploid wheat and *Aegilops tauschii*, and (3) a single mutation in *Triticum aestivum*. Chen et al. (1985) considered this species to be derived from natural hybridization between *T. aestivum* and *T. polonicum* from the cytological analysis of crosses with common wheat and tetraploid species. A dwarfing accession of *T. polonicum* was recently found in Tulufan,

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Xinjiang (Yang et al. 2001; Liu et al. 2002a, b), although it has been suspected that *T. polonicum* is not endemic in western China. Yang et al. (2000a, b) found that gliadin patterns of Xinjiang *T. polonicum* and *T. petropavlovskiyi* were common. *T. petropavlovskiyi* did not show any gliadin polymorphism (Watanabe et al. 2004). Chen et al. (1988) also suggested introgression from *T. polonicum* or some other wild emmer wheat as the mechanism leading to the unusual characteristics of *T. petropavlovskiyi*. Watanabe and Imamura (2002) suggested that *T. petropavlovskiyi* originated as a spontaneous hybrid between *T. aestivum* and *T. polonicum*. Akond and Watanabe (2005) found eight accessions of Portuguese hexaploid wheat, which glume was long and determined by the *P* gene. It was most likely that the *P* gene of the long glumed Arrancada hexaploid wheat was introduced from *T. Polonicum* to *T. aestivum* via a spontaneous introgression or breeding effort. A sterile plant resembling it considerably in terms of spike morphology was collected in Xinjiang. It is considered as F<sub>1</sub> of *T. polonicum* and *Aegilops tauschii*. Yang et al. (1992) suggested that it originated through an independent hybridization event, because of the polonicum-like spike morphology of *T. petropavlovskiyi* and its relatively greater chromosomal differentiation. According to Efremova et al. (2000), *T. petropavlovskiyi* has dominant genes determining at least 13 morphological and physiological characters and a regular bivalent chromosome configuration. They thought that *T. petropavlovskiyi* originated from *T. aestivum* through spontaneous mutation.

In the present study we determined genetic diversity among Xinjiang *Triticum*. We also traced similarity between *T. petropavlovskiyi* with *T. polonicum* from China, Afghanistan, Azerbaijan, Ukraine, Hungary, Romania, Portugal, Cyprus, Turkey, Iraq, Iran, Egypt, Jordan and Ethiopia. The objectives of this study were (1) to assess the genetic diversity among Xinjiang *Triticum* and *T. polonicum* using AFLP assays and (2) to discuss the origin of *T. petropavlovskiyi*.

## Materials and methods

### Accessions of Xinjiang *Triticum* and *Triticum polonicum*

We analyzed 72 landraces of wheat, which included *T. aestivum*, *T. petropavlovskiyi*, *T. compactum*, *T. durum*, *T. turgidum*, and *T. polonicum*. Forty two

Xinjiang *Triticum* accessions were provided from the National Small Grain Collection (NGSC), Aberdeen, Idaho, USA. Seeds of 19 accessions of *T. polonicum*, including 6 Chinese originated *T. polonicum*, were provided by the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Among 11 accessions of *T. petropavlovskiyi*, three were obtained from the Plant Germplasm Institute, Kyoto University, Japan, and six accessions from the N.I. Vavilov Institute of Plant Industry, St. Petersburg, Russia. We obtained one accession from a Chinese colleague, another accession from the late Dr. O.I. Maystrenko, Institute of Cytology and Genetics, the Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia, and another from NGSC. According to the classification of NGSC and IPK, the collection consisted of 39 accessions of *Triticum aestivum* ssp. *aestivum*, 19 accessions of *T. turgidum* ssp. *polonicum*, one accession each of *T. aestivum* ssp. *compactum*, *T. turgidum* ssp. *durum*, and *T. turgidum* ssp. *turgidum*.

Grain colour, growth habit, leaf pubescence and length of glume were recorded for each accession. All the data were taken from the glasshouse and field experiments during 2005 and 2006. Table 1 shows the details of the accessions; the PI number, country of origin, grain colour, growth habit, leaf pubescence and length of glume. Table 3 summarizes plant height, presence of awn, glume awn, leaf pubescence, and spike length of *T. aestivum*, PI 447372 and PI 447384, and 11 accessions of *T. petropavlovskiyi*.

### DNA isolation and AFLP analysis

DNA was isolated from fresh leaves of single plants of accessions of Xinjiang *Triticum* and *Triticum polonicum*, following the Qiagen Dneasy mini kit procedure. AFLP analysis was performed as described by Akond and Watanabe (2005), with partial modifications of the Keygene method (Zabeau and Vos 1993; Vos et al. 1995). The number of monomorphic and polymorphic AFLP fragments was determined for each primer pair. The presence or absence of each fragment was scored as 1 = present, 0 = absent. Genetic similarities based on Jaccard's coefficient (Jaccard 1908) were calculated using the statistical package MVSP-multivariate statistical analysis from Rock Ware Inc. USA (<http://rockware.com>). The genetic similarity matrices were used to generate an UPGMA (un-weighted pair group method of arithmetic means) dendrogram (Sokal and Michener 1958). Principal coordinate analysis (PCO) based on Gower's genetic similarity (Gower 1966) was also performed.

## Results and discussion

### Characteristics of the Xinjiang *Triticum*

Wheat has been cultivated in Xinjiang, China in a great diversity of climates, i.e. severely cold in winter and extremely hot in summer, and this explains its large number of spring and winter varieties. Vernalization requirement was observed at the glasshouse trial in 2005 at Gifu University, Japan. Twenty out of 38 *T. aestivum* accessions have the spring growth habit (Table 1). Hosono (1935) speculated that wheat accessions introduced to China through the Myanmar route were characterized by the red grain colour. The pre-dominance of the red-grain type was not presented in our materials, and the white grain type accounted for 54.71% of the materials from Xinjiang.

Leaf pubescence in wheat has received much attention because of its possible agronomic merits, such as insect resistance (Ringlund and Everson 1968) and drought tolerance (Erdei et al. 1990). We investigated with the naked eye the presence or absence of leaf pubescence on both sides of the penultimate leaf of the main tiller. Almost all accessions with the spring growth habit have pubescent leaves, whereas the accessions with the winter growth habit did not have any leaf pubescence. The length of glume ranged from 18.8 to 22.5 mm in *T. petropavlovskyi*, 7.2 to 10.3 mm in *T. aestivum*, whereas in *T. polonicum* the length was 22 to 38 mm. Considering the caryopsis structure we classified PI 447375 as *T. compactum*, which was listed as *T. aestivum* in NGSC. The profiles of the spikes of Xinjiang *Triticum* and *T. polonicum* are shown in Figure 1.

### AFLP analysis

Seventy two accessions of wheat were analyzed with the four AFLP combinations, namely (i) E-CTG/M-TCT, (ii) E-CTT/M-ACT, (iii) E-TCT/M-ATC, and (iv) E-TTC/M-ACA. These primer combinations were chosen from Akond and Watanabe (2005), due to their suitability in the AFLP assay. Figure 2 shows the banding pattern in a typical AFLP fingerprinting gel produced using E-CTG/M-TCT primer combination. All four primer combinations generated a total of 91 putative loci across all the accessions, among them 56 were polymorphic, which is equivalent to 61.53% of the total number of putative loci (Table 2).

Table 2 shows the selective primers and the number of polymorphic loci scored for each spe-

cies. Eleven accessions of *T. petropavlovskyi* were highly homogeneous, because the frequency of polymorphic loci was lowest among species. Forty four polymorphic loci were found in *T. aestivum* and *T. compactum*, whereas the highest polymorphism was observed in *T. polonicum* from China and other countries. There were no visible bands in several loci in the tetraploid wheat group. Out of these, nine loci were commonly absent in *T. polonicum*, *T. turgidum* and *T. durum*, and were presumed to be D-genome specific loci.

Except for two pairs of *T. aestivum* (PI 447392 – PI 447394, and PI 447387 – PI 447388), the AFLP profiles revealed a wide genetic diversity. On the basis of pair wise similarity coefficients (matrix not shown), the closest pair was PI 447386 and PI 447387, based on similarity coefficients (0.99) among all accessions; both accessions belong to *T. aestivum*. The difference between the two accessions may be due to the minimal intraspecific allelic variation, probably resulting from recombination events. PI 208911, *T. polonicum* from Iraq, was found as the most dissimilar accession (similarity coefficient 0.50) from *T. aestivum* PI 447384 and PI 447372. This dissimilarity may be due to different ploidy levels of those accessions.

A single dendrogram was generated from the UPGMA cluster analysis based on Jaccard's coefficient (Figure 3). As shown in Figure 3, all accessions were classified into four major clusters at the similarity coefficient of 0.75. The similarity coefficient ranged from 0.75 to 0.96 for cluster 1, which clearly separated all accessions of *T. aestivum* and *T. petropavlovskyi*. Cluster 1 contained 12 *T. polonicum* accessions from 12 different countries, 2 Chinese originated *T. polonicum* (TR 18266 and TR 18269), one *T. durum* and one *T. turgidum* accession. Four Chinese originated *T. polonicum* (TR 17455, TR 17453, TR 17459 and TR 18279) and another *T. polonicum* from Azerbaijan (PI 349052) made cluster 2. Cluster 3 consisted of all accessions of *T. petropavlovskyi* and two accessions of *T. aestivum* (PI 447372 and PI 447384) were alienated with them. This suggests a similar genomic constitution of PI 447372, PI 447384 and *T. petropavlovskyi* from the sources from the same geographic area. Cluster analysis showed a higher similarity among the *T. petropavlovskyi* accessions with the similarity value of 0.77 to 0.95. The similar result was found in Akond and Watanabe (2005). Cluster 4 was formed with 36 accessions of *T. aestivum* and 2 accessions of *T. compactum*. The similarity coefficient for this cluster ranged from 0.71 between

**Table 1.** Classification, plantid, country of origin, growth type, grain color, leaf pubescences and glume length of seventy two wheat accessions used in the present study

Accession No.	Plantid	Country of origin	Growth habit	Grain colour	Leaf pubescence (+/-)	Glume length (mm)
1	2	3	4	5	6	7
<i>T. aestivum</i> ssp. <i>aestivum</i>						
PI 31781	Ak-Mecca Bougdai	Xinjiang, China	W	W	-	9.0
PI 31784	Kizil Bougdai	Xinjiang, China	S	R	+	9.2
PI 31788	Kizil Bougdai	Xinjiang, China	S	R	+	8.2
PI 31789	Ak Bougdai	Xinjiang, China	S	W	+	9.0
PI 32038	Kara Bougdai	Xinjiang, China	S	R	+	7.8
PI 101567	-	Xinjiang, China	S	W	+	7.2
PI 435091	-	Xinjiang, China	S	W	+	8.0
PI 435092	-	Xinjiang, China	S	W	+	9.0
PI 447368	Russian No. 83	Xinjiang, China	W	R	-	7.2
PI 447369	Xinjiang No. 2	Xinjiang, China	W	W	-	9.5
PI 447370	ST-84	Xinjiang, China	S	R	+	8.5
PI 447372	ST-86	Xinjiang, China	S	R	+	7.2
PI 447373	ST-87	Xinjiang, China	S	W	+	7.8
PI 447374	ST-88	Xinjiang, China	S	R	+	10.2
PI 447376	ST-90	Xinjiang, China	S	R	+	9.5
PI 447378	Xin Dong No. 3	Xinjiang, China	W	R	-	10.2
PI 447379	Xin Dong No. 2	Xinjiang, China	W	W	-	8.3
PI 447380	Kashigaer baipei	Xinjiang, China	S	W	+	10.0
PI 447381	Haimi 85	Xinjiang, China	S	W	+	7.3
PI 447383	Ili Chun No. 1	Xinjiang, China	S	W	+	10.3
PI 447384	Xin Chun No. 1	Xinjiang, China	S	W	+	7.5
PI 447385	Qitai Chun # 4	Xinjiang, China	S	W	+	9.0
PI 447386	Turpan Shan Yueh Huang C	Xinjiang, China	S	W	+	7.3
PI 447387	Toksun Spring No. 1	Xinjiang, China	S	W	+	10.0
PI 447388	Toksun Spring No. 2	Xinjiang, China	S	R	-	9.2
PI 447389	Chanji Bai Dong Mai	Xinjiang, China	W	W	-	10.0
PI 447390	Turpan Bai Dong Mai	Xinjiang, China	W	W	-	12.0
PI 447391	Kuerle Bai Dong Mai	Xinjiang, China	W	W	-	7.2
PI 447392	Kuerle Balibasi	Xinjiang, China	W	W	-	9.2
PI 447393	Kashigaer Bakefuke	Xinjiang, China	W	W	-	8.8
PI 447394	Xin Dong No. 1	Xinjiang, China	W	R	-	9.0
PI 447395	Xin Dong No. 2	Xinjiang, China	W	W	-	7.8
PI 447396	Xin Dong No. 3	Xinjiang, China	W	R	-	8.0
PI 447397	Xin Dong No. 4	Xinjiang, China	W	W	-	9.2
PI 447398	Xin Dong No. 5	Xinjiang, China	W	W	-	8.0
PI 447399	Xin Dong No. 6	Xinjiang, China	W	W	-	8.2
PI 447401	Xin Dong No. 11	Xinjiang, China	W	W	-	9.0
PI 524983	Hei Mang Dong Mai	Xinjiang, China	W	R	-	8.5
<i>T. aestivum</i> ssp. <i>compactum</i>						
PI 447375	ST-89/ Big Head	Xinjiang, China	S	R	+	7.2
PI 447377	ST-91/ Big Head	Xinjiang, China	S	R	+	7.5
<i>T. turgidum</i> ssp. <i>durum</i>						
PI 447421	ST-33	Xinjiang, China	S	R	+	12.0
<i>T. turgidum</i> ssp. <i>tugidrum</i>						
PI 32039	1584a	Xinjiang, China	S	W	+	10.3
<i>T. turgidum</i> ssp. <i>polonicum</i>						
TR 17453	-	China	S	W		28.5
TR 17455	-	China	S	W		29.2
TR 17459	-	China	S	W		22.0
TR 18266	-	China	S	W		27
TR 18269	-	China	S	R		30.5

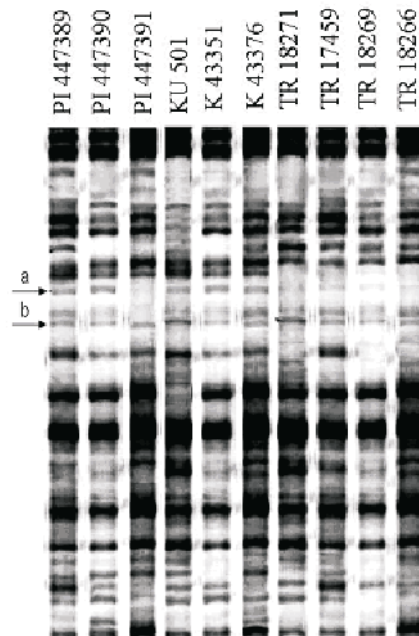
1	2	3	4	5	6	7
TR 18271	–	China	S	W	+	26.0
PI 245663	3002	Afghanistan	S	W	–	29.0
PI 349052	WIR 42758	Azerbaijan	S	W	–	17.5
PI 29447	Assyrian Rye	Ukraine,	S	W	–	31.3
PI 272564	1-1-3496	Pest, Hungary	S	W	–	28.0
PI 306548	2939	Romania	S	R	–	38.0
PI 167622	Mika	Balikesir, Turkey	S	W	–	24.0
PI 208911	Sin El-Pheel	Iraq	S	W	–	23.0
PI 210845	7959	Iran	S	W	–	28.3
PI 352489	T-1515	Cyprus	S	R	–	36.0
PI 366117	D-10	Egypt	S	W	–	27.0
PI 223171	Tafeelih Riti	Jordan	S	R	+	24.5
CItr 14803	ELS 6404-123-1	Tigre, Ethiopia	S	R	+	24.0
PI 191810	Arrancada	Aveiro, Portugal	S	W	+	27
<i>T. petropavlovskyi</i>						
KU501	–	Xinjiang, China	S	R	+	23.5
KU502	–	Xinjiang, China	S	R	+	21.3
KU503	–	Xinjiang, China	S	W	+	20.0
k43351	–	Xinjiang, China	S	R	+	22.5
k43376	–	Xinjiang, China	S	W	+	22.5
k44126	–	Xinjiang, China	S	W	+	20.2
k51763	–	Xinjiang, China	S	R	+	21.5
k51764	–	Xinjiang, China	S	R	+	22.5
k51766	–	Xinjiang, China	S	R	+	18.8
To Mai Chi	–	Xinjiang, China	S	R	+	23.5
O.I.M.	–	Xinjiang, China	S	R	+	18.8

Note: growth habit - Spring (S), Winter (W); grain color - Red (R), White (W); leaf pubescences - present (+), absent (–)



**Figure 1.** Spike profiles of Xinjiang *Triticum* and *T. polonicum*. Left to right: *T. compactum* PI 447377(1), *T. aestivum* PI 31781(2), *T. aestivum* PI 447372(3), *T. aestivum* PI 447384(4), *T. petropavlovskyi* K 43351(5), *T. petropavlovskyi* KU 502(6), *T. polonicum* TR 18271(7), *T. polonicum* TR 17459 (8), *T. polonicum* PI 223171(9), *T. turgidum* PI 32039 (10) and *T. durum* PI 447421(11). *T. aestivum*; PI 447372(3), PI 447384(4), and *T. petropavlovskyi*; K 43351(5) KU 502(6) resemble *T. petropavlovskyi* in respect of spike structure, i.e. presence of awn, glume awn.

PI 447381 and PI 447375 to 0.99 between PI 447386 and PI 447387. At least four sub-clusters were formed within cluster 4. The accessions with the spring and winter growth type were grouped together with some exceptions in those sub-clusters.



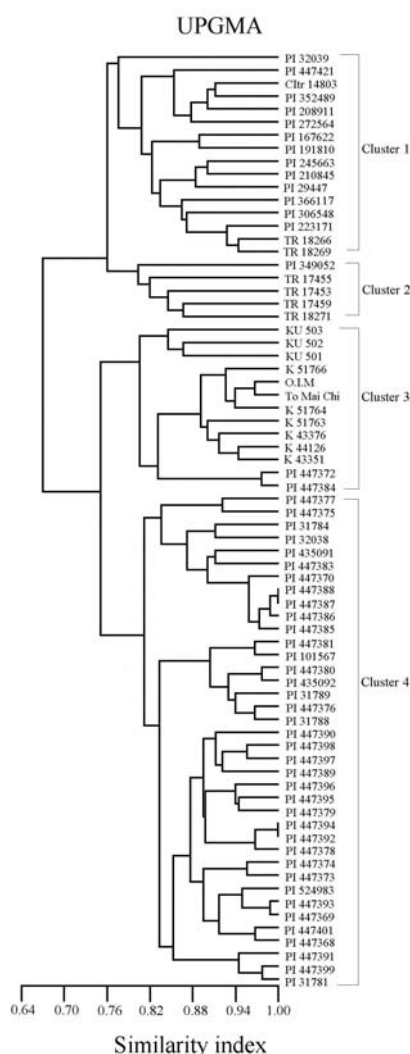
**Figure 2.** A typical AFLP fingerprinting gel produced using E-CTG/M-TCT primer combinations. Arrows indicates (a) polymorphic loci and (b) monomorphic loci

The *T. petropavlovskyi* landrace group was more related to the group of Chinese *T. polonicum* and 2 accessions of *T. aestivum* (PI 447372 and PI 447384). For the pair wise similarity Chinese

**Table 2.** Primer combinations and number of polymorphic AFLP loci

Primer pair*	Putative loci among accessions		Polymorphic loci within subspecies and long glumed accessions			
	number of loci	polymorphic loci	<i>T. aestivum</i>	<i>T. petropavlovskyi</i>	<i>T. polonicum</i> (China)	<i>T. polonicum</i> , (other countries)
E-CTG/M-TCT	25	17	13	12	11	15
E-CTT/M-ACT	23	13	11	8	10	10
E-TCT/M-ATC	22	11	11	6	10	10
E-TTC/M-ACA	21	15	10	6	14	14
Total	91	56	44	32	45	49

\*E=5'-GACTGCGTACCAATTC-3', M=5'-CTACTCAGGACTCATT-3'

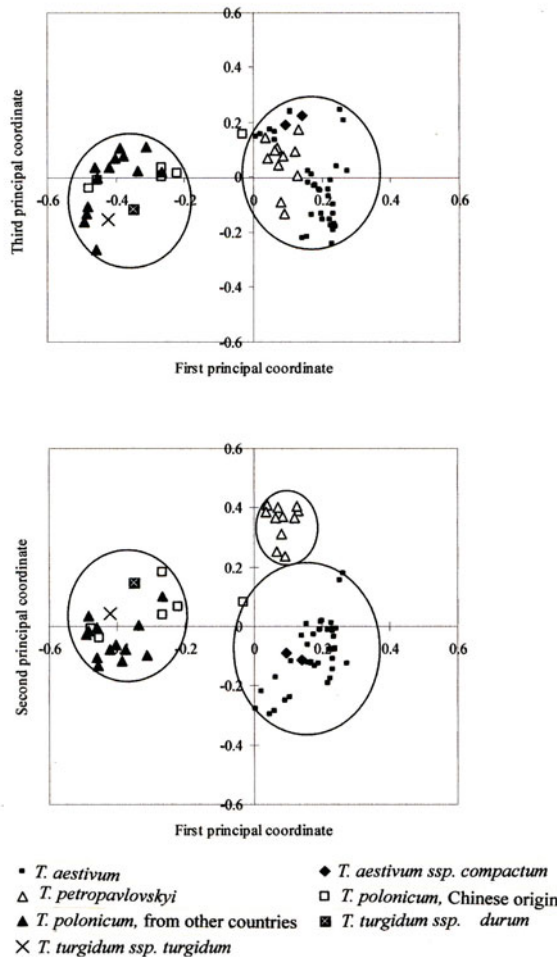
**Figure 3.** UPGMA dendrogram based on Jaccard's coefficient

*T. polonicum* TR 18279 shows the value of 0.80 for the similarity coefficient with accession K51764 of *T. petropavlovskyi* and Chinese *T. polonicum* TR 17455 shows the value of 0.79 for the similarity coefficient with the

*T. petropavlovskyi* accession, To Mai Chi. The lowest similarity was found between TR 18266 and K51766 and also between TR 18271 and KU501 at the similarity coefficient of 0.60. Overall the Chinese originated *T. polonicum* were found to be closer than that of *T. polonicum* from other countries except for PI 349052 from Azerbaijan. Among all accessions of *T. polonicum* it was found that *T. polonicum* was more diverse than *T. aestivum* and *T. petropavlovskyi* of clusters 3 and 4.

Principal coordinate analysis (PCO) is one of the multivariate approaches to grouping, based on the similarity coefficients or variance-covariance among the traits of the entries. It is expected to be more informative about differentiation among major groups, while cluster analysis provides a higher resolution among closely related populations. PCO based on Gower's similarity coefficient (Gower 1966) showed the relationship among the accessions in two dimensional spaces. Up to 47.34% of the total variation can be explained by the first three eigenvectors, which accounted for 26.37, 13.58, and 7.39% respectively.

As shown in Figure 4, two PCO-plots were generated based on the first-second eigenvectors and first-third eigenvectors. The figure based on the first-second eigenvectors revealed three distinct groups, hexaploid (*T. aestivum* and *T. petropavlovskyi*) and tetraploid (*T. polonicum*). All normal glumed *T. aestivum* and *T. compactum* were found within group 1. Group 2 contained 11 accessions of *T. petropavlovskyi* and group 3 contained tetraploids, *T. polonicum*, one *T. turgidum* and another *T. durum*. The figure based on the first-third eigenvectors separated hexaploid and tetraploid wheats and two distinct groups were found. This accounted for 33.76% of the total variation. Four accession of Chinese *T. polonicum* of cluster 2 and 11 accessions of *T. petropavlovskyi* of cluster 3 appeared closer to *T. aestivum*. There is no suggestion of separate groups among



**Figure 4.** Groups derived from principal coordinate analysis (PCO) based on Gower's coefficient

*T. aestivum* and *T. petropavlovskyi* due to the overlap. *Triticum polonicum* from other countries appeared in the most left quadrants of the PCO-plot, in good accordance with the result of the UPGMA cluster analysis. On the contrary, the genetic relationship among geographically related populations was visualized better by PCO analysis.

#### Origin of *T. petropavlovskyi*

Since the first discovery of *T. petropavlovskyi* in Xingjian, China in 1948 it was thought that it may have originated through an independent interspecific hybridization event (Yang et al. 1992). In turn, Chen et al. (1988) and Chen et al. (1985) suggested introgression into *T. aestivum* from *T. polonicum* or some other wild emmer wheat as the mechanism leading to the long glumed characteristic of *T. petropavlovskyi*. In spite of some cytogenetic studies of these Chinese accessions, none of them has presented a completely convincing case for independent interspecific hybridization. We did not define the D-genome alleles in the hexaploid *T. petropavlovskyi* in the present study. Nonetheless, on the basis of the genetic similarity estimates from the AFLPs, it may be noted that *T. petropavlovskyi* was more closely related to the Chinese accessions of *T. polonicum* than *T. polonicum* from other countries. Visible bands

**Table 3.** Plant height, presence of glume awn, awn, leaf pubescences and spike length of *T. aestivum* (PI 447372 and PI 447384), and 11 accessions of *T. petropavlovskyi*

Accession number	Plant height (cm)	Glume awn (+/-)	Awn(+/-)	Spike length (cm)	Leaf pubescences (+/-)
PI 447372	138	+	+	13.2	+
PI 447384	131	+	+	14.2	+
KU501	125	+	+	18.2	+
KU502	134	+	+	15.0	+
KU503	118	+	+	14.8	+
k43351	141	+	+	16.2	+
k43376	144	+	+	14.4	+
k44126	127	+	+	15.0	+
k51763	121	+	+	15.5	+
k51764	132	+	+	18.0	+
k51766	120	+	+	15.5	+
To Mai Chi	116	+	+	14.0	+
O.I.M.	105	+	+	15.2	+

Note: Glume awn, awn and leaf pubescences - present (+), absent (-)

were commonly absent in six loci of the Chinese *T. polonicum*, which were found absent in almost all of the *T. petropavlovskyi* accessions. This result reduced the probability of independent allopolyploidization event in *T. petropavlovskyi*.

UPGMA clustering and PCO grouping showed that *T. petropavlovskyi* was more genetically similar to Chinese *T. polonicum*. Two accessions of *T. aestivum*, PI 447372 and PI 447384, which were grouped with *T. petropavlovskyi* in UPGMA clustering, resemble *T. petropavlovskyi* in respect of spike structure, i.e. the presence of awn, glume awn and the presence of leaf pubescence (Table 3).

*T. petropavlovskyi* is characterized by having a glume awn or awn-like appendage on the glume. Watanabe and Imamura (2002) suggested that *T. petropavlovskyi* originated from the natural hybridization between *T. aestivum* that has an awn-like appendage on the glume and *T. polonicum*. We found a high level of similarity among several *T. petropavlovskyi* and *T. polonicum* of China, although they are different in ploidy. Between the two sub-species the similarity coefficient ranged from 0.60 to 0.80. Findings of this study reduced the probability of the origin of *T. petropavlovskyi* by an independent allopolyploidization event or single mutation in *Triticum aestivum* and suggested a greater degree of gene flow between *T. aestivum* and *T. polonicum* leading to *T. petropavlovskyi*. It is most likely that the *P*-gene of the *T. petropavlovskyi* hexaploid wheat was introduced from *T. polonicum* to *T. aestivum* via spontaneous introgression or breeding effort.

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