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Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance

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Abstract The genetic identity of eight wheat (*Triticum aestivum* L.) accessions maintained in the Gatersleben genebank and regenerated up to 24 times was studied by using wheat microsatellite markers (WMS). It was demonstrated that WMS can be used to analyze bulks of seeds stored more than 50 years in a seed reference collection at room temperature. No contamination due to foreign pollen or incorrect handling during the multiplication cycles was discovered. For one accession (TRI 4599) genetic drift was observed, whereas for TRI 249 a heterogenous situation for two markers was maintained over the years. We were able to show that microsatellites can be used as a simple and reliable marker system for the verification of the integrity and genetic stability of genebank accessions.

Key words *Ex-situ* conservation · Genebank collection · Long term storage · Mikrosatellites · Wheat

Introduction

The anticipated growth of the human population demands an extended global food production by increasing the agricultural productivity. Beside higher farming inputs the most viable approach is the genetic improvement of crops. Therefore, new sources of genetic variation need to be provided continuously. In genebanks worldwide about six millions accessions of cultivated crops are conserved, including about 748,500 wheats (FAO 1996a, b). One of the main challenges for genebanks is the maintenance of the genetic integrity of accessions. The contamination by foreign pollen or incorrect handling during multiplication may affect the genetic identity of the material. Molecular methods assessing genetic variation at the DNA level will be useful in proving the purity of genebank accessions after long term maintenance.

Previous work in bread wheat has demonstrated that microsatellites or simple sequence repeats (SSRs) show a higher level of polymorphism than any other marker system (Röder et al. 1995; Plaschke et al. 1995; Bryan et al. 1997). Röder et al. (1998) recently showed that microsatellites are genome-specific markers and appear to be evenly distributed over the wheat genome. Therefore, they are highly suitable as genetic markers in wheat for mapping agronomically important genes (Korzun et al. 1997a, 1998), studying the genetic diversity of bread wheat and related species (Plaschke et al. 1995; Fahima et al. 1998) and verifying the identity of cytogenetic stocks (Korzun et al. 1997b).

In the study described here the genetic identity of wheat accessions of the Gatersleben genebank regenerated up to 24 times during the last 50 years is studied by applying wheat microsatellite markers. Results of a comparison of DNA extracted from grains of both the first and the last multiplication cycles are presented.

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Materials and methods

Plant materials and DNA isolation

Eight wheat (*Triticum aestivum* L.) accessions differing in their frequency of multiplication were randomly selected out of the Gatersleben genebank wheat collection consisting of about 17,000 accessions in total. From each accession a sample of grains and complete spikes are deposited as vouchers when they are grown initially. Although the samples are stored at room temperature and, therefore, have lost their germinability, it is still possible to extract DNA for comparative studies. Living seed materials originated from the most recent regeneration are stored in the seed storage at a temperature of 0°C. The geographical origins, the morphological groups, the frequencies of multiplication, varying from 5 to 24, and the years of the first and last regenerations of the analyzed wheat accessions are given in Table 1. Moreover, three additional accessions known to have originated from the same collection

Table 1 Origins, morphological groups, years of multiplication and regeneration frequencies of the wheats (*Triticum aestivum* L.) analyzed

Catalogue number Gatersleben	Variety	Origin	Morphological group	Years of first and last multiplication	Regeneration frequency
TRI 11742	Landrace	Pakistan	var. *aestivum*	1978, 1997	5
TRI 12922	'Chinese Spring'	China	var. *lutinflatum* (Flaksb.) Mansf.	1979, 1992	6
TRI 249	'Janetzkis Frühe Kreuzung'	Germany	var. *lutescens* (Alef.) Mansf.	1946, 1995	11
TRI 2292	Landrace	Greece	var. *erythroleucon* (Körn.) Mansf.	1952, 1995	11
TRI 4599	Landrace	Albania	var. *aureum* (Link) Mansf.	1952, 1996	15
TRI 3342	Landrace	China	var. *subsardoum* (Vav. et Jacubz.) Mansf.	1951, 1995	16
TRI 1634	Landrace	Albania	var. *ferrugineum* (Alef.) Mansf.	1948, 1996	17
TRI 2519	Landrace	Tibet	var. *villosum* (Alef.) Mansf.	1950, 1996	24
TRI 1646[a]	Landrace	Albania	var. *aestivum*	1948, 1979	16
TRI 1648[a]	Landrace	Albania	var. *lutescens* (Alef.) Mansf.	1948, 1983	16
TRI 4591[a]	Landrace	Albania	var. *aestivum*	1952, 1983	10

[a] Originated from the same collection sample as TRI 4599 and investigated using only one molecular marker

Table 2 Fragment sizes, chromosomal locations and numbers of detected alleles for the microsatellites used

Microsatellite marker	Fragment size (in bp)	Chromosome location	Number of detected alleles
WMS3[a]	87–92	3D	4
WMS186[a]	96–140	5A	8
WMS261[a]	164–200	2D	6
WMS357[a]	114–120	1A	4
WMS437[a]	89–126	7D	7
WMS445[a]	181–186	2A	3
WMS619[b]	132–166	2B	5
WMS631[b]	182–194	7A	4
WMS680[b]	105–118	6B	2

[a] Sequence information on the WMS is given in Röder et al. (1998)
[b] Sequence information can be obtained from Dr. M. Röder, IPK, Gatersleben

sample as TRI 4599 were investigated using one of the nine molecular markers only.

Five grains of each accession derived from the first and last regeneration cycles were pooled and ground in a microhammer mill. The DNA was extracted according to the procedure described by Plaschke et al. (1995). For TRI 249 DNA was also extracted from 20 single grains of both the 1946 and 1995 harvests.

Microsatellite analysis

The development of the wheat microsatellite markers (WMS) has been described by Röder et al. (1995, 1998). Nine primer pairs of wheat WMS with different chromosomal locations were chosen for analysis. Microsatellites designation, fragment sizes, chromosome location of the amplified loci, and number of alleles detected for the polymerase chain reaction (PCR) are shown in Table 2. Sequence information on the WMS is given in Röder et al. (1998) or can be obtained from Dr. M. Röder, IPK Gatersleben. PCR reactions and fragment detection were performed as described by Röder et al. (1995) and Plaschke et al. (1995).

Results

With the nine microsatellites located on nine different chromosomes a total of 43 alleles were discovered. The number of alleles per locus ranged from 2 (WMS680) to 8 (WMS186). On average 4.8 alleles were detected.

Table 3. Number of alleles amplified with nine WMS per accession and year of harvest

Catalog number Gatersleben	Years of multiplication	WMS								
		3	186	261	357	437	445	619	631	680
TRI 11742	1978	1	1	1	1	1	1	1	1	1
	1997	1	1	1	1	1	1	1	1	1
TRI 12922	1979	1	1	1	1	1	1	1	1	1
	1992	1	1	1	1	1	1	1	1	1
TRI 249	1946	1	2	1	1	2	1	1	1	1
	1995	1	2	1	1	2	1	1	1	1
TRI 2292	1952	1	1	1	1	1	1	1	1	1
	1995	1	1	1	1	1	1	1	1	1
TRI 4599	1952	2	1	2	1	2	–	1	1	1
	1996	1	1	1	1	1	1	1	1	1
TRI 3342	1951	1	1	1	1	1	1	1	1	1
	1995	1	1	1	1	1	1	1	1	1
TRI 1634	1948	1	1	1	1	1	1	1	1	1
	1996	1	1	1	1	1	1	1	1	1
TRI 2519	1950	1	1	1	1	1	–	1	1	1
	1996	1	1	1	1	1	1	1	1	1

As shown in Table 3, in 65 of the 72 possible comparisons of the alleles (8 accessions × 9 microsatellites) detected in the samples from the first and the last multiplication cycles of each accession one allele was detected in each sample showing identity between the two seed generations analyzed. One example showing the fragments detected from seeds of the first and last regeneration of the analyzed wheat accessions amplified with WMS186 is given in Fig. 1. The figure shows also one of the exceptions obtained for TRI 249. In both years two alleles were discovered which were, however, identical. Also, two identical alleles were obtained for TRI 249 using WMS437. Based on this result 20 single grains of both regeneration cycles were investigated using WMS186 in order to find out whether the accession was heterozygous or heterogenous at that locus. Unfortu-

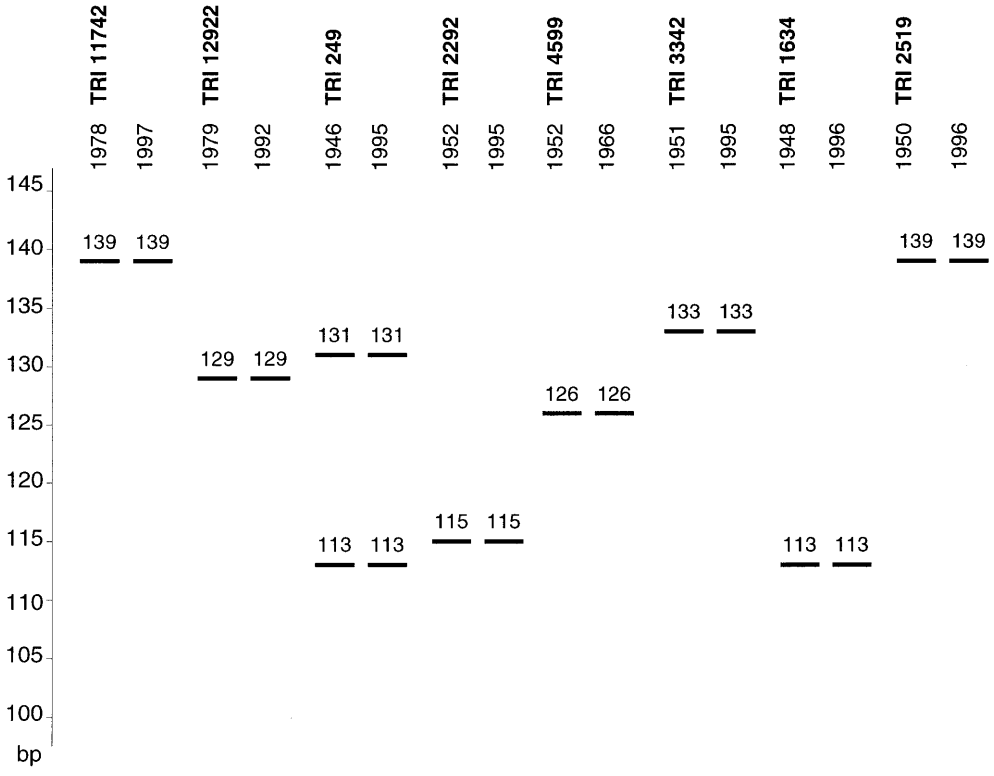

Fig. 1 WMS186-amplified PCR fragments detected from seeds of the first and last multiplications of eight wheat accessions

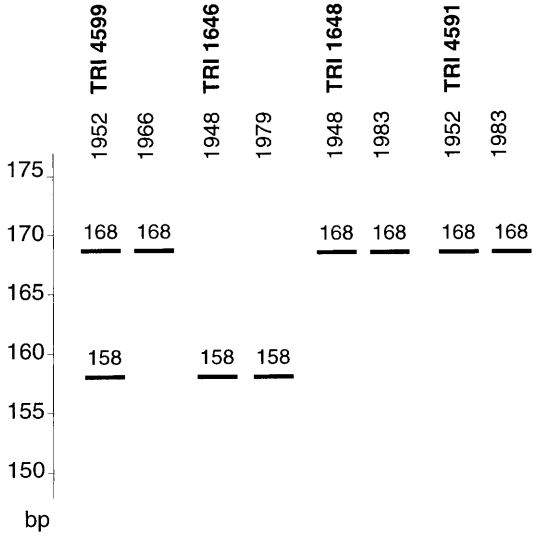

Fig. 2 PCR fragments amplified by WMS261 of the first and last multiplications of 4 wheat accessions originated from the collection number 1456

nately, using the grains of the 1946 harvest we were unable to obtain any amplification of the DNA of the single grains. For the fresh seeds harvested in 1995, however, we found that all genotypes were homozygous, amplifying one single fragment of 113 or 130 bp in a ratio of 9 : 11, respectively.

Three deviations were recovered while investigating TRI 4599 (Table 3). Whereas two alleles were always detected when analyzing the seeds of the 1952 harvest, after 15 multiplications, in 1996, only one of the two remained. The additional analysis of three extra accessions taken from the same collection sample as TRI 4599 showed that for WMS261 in both samples of each of the accessions only one of the two alleles detected for TRI 4599 in 1952 is present (Fig. 2).

With the DNA extracted from seeds of the first regeneration in 1950 and 1952 of the wheat accessions TRI 2519 and TRI 4599, respectively, no fragments were amplified by WMS445. Therefore, WMS445 seems to be more sensitive to the quality of DNA than all other microsatellites used in the present study.

Discussion

Simple sequence repeats commonly known as microsatellites, have been shown to be a very useful marker type for wheat. Given the results obtained from gene mapping or genetic diversity studies we used that type of molecular marker to analyze the purity of *ex-situ* genebank collections and were able to demonstrate how new techniques may be employed in combination with germplasm collected more than 50 years ago.

It is known that wheat microsatellites are highly polymorphic revealing a high number of alleles. The average of 4.8 alleles per WMS obtained in the present study is comparable to 6.2 described by Plaschke et al. (1995) studying the genetic diversity of 40, mainly European, wheat cultivars. The degree of polymorphism even increases using the wild relatives of bread wheat. In *Triticum dicoccoides,* the tetraploid progenitor of cultivated

wheat, the average number of alleles per WMS was observed to be about 10 with a maximum of 18 (Fahima et al. 1998). Similar frequencies were obtained for diploid wheat species (Korzun, unpublished data).

Our analyses of stocks multiplied up to 24 times showed a high degree of identity that results from the high quality of the genebank management in Gatersleben. No contamination resulting from cross pollination or erroneous handling during harvesting, threshing or labeling was detected. We were able to show, however, that genetic drift had occurred in accession TRI 4599. For three WMS two alleles were detected in the bulk of the original seeds from the 1952 harvest, whereas in 1996 only one allele remained. TRI 4599 was extracted from a seed sample collected in 1941 in Albania having the collection number 1456. This sample was heterogenous and, therefore, after subsequent regenerations divided into five accessions (TRI 1646, TRI 1648, TRI 4591, TRI 4599) belonging to three different morphological groups (Table 1). Whereas in 1952 TRI 4599 was still not a pure line it seems to be more homogenous now after 15 times regeneration.

Another example of heterogenity was shown for TRI 249, an old German winter wheat variety named 'Janetzkis Frühe Kreuzung' bred in the early 1930s. Here, however the heterogenous situation was still evident for two WMS after 11 multiplication cycles. The explanation for the maintenance of this heterogenity may be that both markers seem not to be linked to genes sensitive to genetic shift or drift.

Comparable studies using germplasm collections are known only from oat, which is also a self-pollinating cereal species. Using storage proteins Steiner et al. (1997) investigated seven seed samples maintained at three different sites. Whereas a cross fertilization between the tested oat lines could be excluded by the electrophoresis patterns several types of contamination were detected, most probably a result of incorrect handling during the regeneration cycles. We confirm the conclusion of Steiner et al. (1997) that the maintenance of genetic integrity of genebank accessions requires a most careful handling of passport data, regeneration and storage. The introduction of new molecular methods will improve genebank management in the future. DNA fingerprinting techniques should provide simple and reliable ways for the identification of genebank accessions and the verification of their stability.

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