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Short Communication

Gliadin electrophoretic analysis of the genetic integrity of wheat (*Triticum aestivum* L.) accessions after frequent seed reproductions

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Abstract

A standard electrophoretic method for wheat cultivar identification was used on single seeds to analyse the genetic integrity of 11 wheat (*Triticum aestivum* L.) accessions after up to 24 seed reproductions in the Gatersleben genebank. It was clearly demonstrated that the gliadin pattern of single seeds can be used to analyse the genotype composition of wheat accessions. Stability of electrophoretic banding patterns was detected in eight accessions. Very week genetic drift was observed in three accessions. Our investigations confirm experiences of the successful utilisation of protein markers for cultivar verification and genetic integrity testing and demonstrate the high standard of wheat accessions maintenance in the Gatersleben genebank.

Introduction

One of the main objectives of *ex-situ* conservation is to maintain accessions without changing their genetic constitution. For material conserved as seeds, there is a periodic need to regenerate a sample (Konarev et al. 1995; Hodgkin 1997; Steiner et al. 1997; Börner et al. 2000). The methods used for the maintenance of genebank collections are designed to minimise the risk of changes occurring through contamination, selection, random drift or mutation. For regeneration the material has to be grown in ways that prevent genetic drift or shift by using a population size which is large enough and under good seed production conditions. The control of genetic stability during reproductions of an original sample is usually carried out by using morphological characters which, however, have some limitations (Konarev et al. 1996, 2002; Hodgkin 1997; Wrigley and Batey 1999).

Genebank accessions frequently represent a mixture of genotypes. The identification of these genotypes is extremely difficult. The phenotypic characterisation is often not sufficient for that. Therefore, molecular methods assessing genetic variation may be a useful tool for inspecting the genetic integrity of genebank accessions after long term maintenance (Vvedenskaya et al. 1993; Konarev et al. 1996; Hodgkin 1997; Börner et al. 2000; Chebotar et al. 2003).

Protein markers are successfully used for increasing the efficiency of the utilisation of plant genetic resources at the N.I. Vavilov Institute, St. Petersburg, Russia since 1969. Using molecular approaches the following aspects of plant genetic resources were studied: (a) estimating the structure of biodiversity (intra- and interspecific

relationships, genome analysis); (b) identification and registration of genetic diversity and establishing data bases and catalogues based on protein formulae; (c) identification of duplicates; (d) development of core collections; (e) control of genetic integrity; (f) control of authorship rights for genebanks (Konarev et al. 1979, 1996, 2002). The present level of electrophoretic technique of seed proteins offered possibilities to identify varieties for a large group of crops. Wheat cultivar identification using standard electrophoretic methods for gliadin storage proteins, elaborated in the frame of International Seed Testing Association (ISTA), are valid since 1983 (Anonymous 1996). The pattern of gliadin bands (electropherogram) is related to the genetic constitution and can be considered as a 'fingerprint' of the cultivar or accession. The 'fingerprints' can be used to identify unknown samples and mixtures by single seed analysis (Anonymous 1996).

The experiments presented here were performed to investigate the utilisation of gliadin polymorphism of bread wheat for estimating the degree of genetic identity of early and recent reproductions of the same accessions after numerous multiplication cycles. The results are compared with data obtained earlier by using DNA markers (Börner et al. 2000).

Materials and methods

Eleven wheat (*Triticum aestivum* L.) accessions differing in their frequency of being reproduced (Table 1) were selected from the Gatersleben genebank collection. The number of multiplications is identical with the number of regenerations, i.e., for each seed regeneration seed material from the previous regeneration was used. The origins and morphological groups were described earlier (Börner et al. 2000). From each accession 25–30 seeds, deposited as vouchers when they were grown initially and 25–30 seeds from the most recent regeneration were investigated.

The standard reference method of gliadin PAAG (polyacrylamide) electrophoresis was used (Konarev et al. 1979; Anonymous 1996). Wheat gliadins were extracted from crushed single seeds with 40 μL aqueous ethanol and separated by PAAG at pH 3.2. After electrophoresis gels were stained with 0.075% Coomassie G-250 and photographed or scanned. Electrophoretic patterns were written down in the form of protein formula by means of etalon pattern (Konarev et al. 1979, 1996) as α-, β-, γ- and ω-gliadin (prolamin) fractions.

The protein formula includes the designations of pattern zones in letters and number of positions within these zones, which are occupied by components and subcomponents of prolamins for a given source (Konarev et al. 1979, 1996).

Results

The protein formula of single seeds obtained from the original sample and the last reproduction are given in Table 1. Monotypic pattern (one gliadin pattern) identical for both samples were obtained for accessions TRI 1634, TRI 1648, TRI 2292, TRI 3342 and TRI 12922, whereas two matching patterns (types I and II) were detected in accessions TRI 2519 and TRI 11742 with comparable frequencies of occurrence. A slight shift in frequency of pattern type was found in accession TRI 4591.

Two protein patterns were also found in the original sample of TRI 249 mainly represented by type I. The rare type pattern II was detected with a frequency of less than 10%. After 11 reproduction cycles the type I genotypes were still dominating. The second genotype appeared with a frequency slightly higher than 10%. In addition, with a frequency of about 30%, seeds with pattern type III were identified missing the components 3_3 and 3 in β and γ zones, respectively.

Accession TRI 1646 was monotypic for the gliadin pattern in the original sample (26 seeds). A second pattern type appeared with a frequency of about 10% in the sample of the most recent regeneration.

Finally for accession TRI 4599 three pattern types were identified in the sample originated from 1952. Type I was dominating (70%), whereas types II and III were minor ones having equal frequencies (15%). Type III pattern was not detected in the sample of the latest regeneration.

Discussion

Molecular markers are widely used for controlling the dynamics (changes) of genotype composition of wild growing populations and varieties of

Table 1. Distribution of gliadin banding patterns in wheat (*Triticum aestivum* L.) accessions detected from single seeds obtained from the first and most recent reproductions.

Cat. No. Gatersleben	Regeneration frequency	Years of the first and last reproduction	Pattern type	Protein (gliadin) formulae α	β	γ	ω
TRI 11742	5	1978	I	$3\ 6_1\ \underline{7}_1\bar{7}_2$	$2\ 3_2\ 4\ 5_1\bar{\bar{5}}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$2\ \bar{4}_2\ 6_2\bar{6}_3\ 7_1\ 8_1\bar{8}_2\ \underline{9}_2$
			II	$3\ 6_1\ \underline{7}_1 7_2$	$2\ 3_2\ \bar{4}\ 5_1\bar{\bar{5}}_2$	$2_2\ \underline{3}\ \underline{4}$	$\bar{2}\ \bar{4}_2\ 6_2\bar{6}_3\ 7_1\ 8_1\bar{8}_2\ 9_2$
		1997	I	$3\ 6_1\ \underline{7}_1\bar{7}_2$	$2\ 3_2\ \bar{4}\ 5_1\bar{5}_2$	$\bar{\bar{2}}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{2}\ \bar{4}_2\ 6_2\bar{6}_3\ 7_1\ 8_1 8_2\ 9_2$
			II	$3\ 6_1\ \underline{7}_1\bar{7}_2$	$2\ 3_2\ \bar{4}\ 5_1\bar{5}_2$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$2\ 4_2\ 6_2\ 6_3\ 7_1\ 8_1 8_2\ \underline{9}_2$
TRI 12922	6	1979	I	$\bar{2}\ \underline{4}\ \underline{6}_1\ 7_1 7_2$	$\underline{2}\ 3_2\ \underline{4}\ 5_2$	$1\ 2_3\ \underline{3}$	$4_1 4_3\ 5\ 8_2\ \underline{9}_2$
		1992	I	$\bar{2}\ \underline{4}\ \underline{6}_1\ 7_1 7_2$	$\underline{2}\ 3_2\ \underline{4}\ 5_2$	$1\ 2_3\ \underline{3}$	$4_1 4_3\ 5\ 8_2\ \underline{9}_2$
TRI 4591	10	1952	I	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$\bar{2}\ 4_2\ 6_1\underline{6}_2\bar{\bar{6}}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
			II	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ 4\ 5_1$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{2}\ 4_2\ \bar{\bar{6}}_1\underline{6}_2\bar{\bar{6}}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
		1983	I	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{2}\ 4_2\ \bar{\bar{6}}_1\underline{6}_2\bar{\bar{6}}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
			II	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ 4\ 5_1$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$2\ 4_2\ \bar{\bar{6}}_1\underline{6}_2\bar{6}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
TRI 2292	11	1952	I	$3\ 5\ 6_1\ \underline{7}_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ \underline{4}\ \bar{5}_2$	$2_1 2_3\ \underline{3}\ 4$	$\underline{4}_1 4_3\ 6_2\ \bar{\bar{8}}_2\ \underline{9}_2$
		1995	I	$3\ 5\ 6_1\ \underline{7}_1 7_2$	$1\ \underline{2}\ 3_2\ \underline{4}\ 5_2$	$2_1 2_3\ \underline{3}\ 4$	$\underline{4}_1 4_3\ 6_2\ \bar{\bar{8}}_2\ \underline{9}_2$
TRI 249	11	1946	I	$\underline{6}_1\ \underline{7}_1 7_2$	$2\ 3_2 3_3\ 4\ 5_2$	$2_1 2_3\ \underline{3}\ \underline{4}$	$\bar{2}\ \bar{\bar{3}}\ 6_2\underline{6}_3\ \bar{\bar{8}}_2\ \bar{\bar{9}}_2$
			II	$\underline{6}_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2 3_3\ 4\ 5_2$	$2_1 2_3\ 3\ 4$	$\bar{\bar{3}}\ 6_2\ 6_3\ \underline{8}_2\ \underline{9}_2$
		1995	I	$\underline{6}_1\ \underline{7}_1 7_2$	$2\ 3_2 3_3\ 4\ 5_2$	$2_1 2_3\ \underline{3}\ \underline{4}$	$\bar{2}\ \bar{\bar{3}}\ 6_2\ 6_3\ \bar{\bar{8}}_2\ \bar{\bar{9}}_2$
			II	$\underline{6}_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2 3_3\ 4\ 5_2$	$2_1 2_3\ 3\ 4$	$\bar{\bar{3}}\ 6_2\ 6_3\ \underline{8}_2\ \underline{9}_2$
			III	$\underline{6}_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2\ \underline{4}\ 5_2$	$2_1 2_3\ 4$	$3\ 6_2\ 6_3\ \bar{\bar{8}}_2\ 9_2$
TRI 4599	15	1952	I	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$\bar{1}\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{\bar{2}}\ 4_2\ 6_1\underline{6}_2\bar{6}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
			II	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$\bar{1}\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\underline{2}_2\ \underline{3}\ \underline{4}$	$\bar{2}\ \underline{4}_2\ \underline{6}_2\bar{6}_3\ 7_1\ 8_1 8_2\ 9_2$
			III	$\underline{6}_1\ 7_1\bar{7}_2$	$\bar{1}\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{\bar{2}}\ \underline{4}_2\ \underline{6}_2\bar{6}_3\ 7_1\ 8_1 8_2\ 9_2$
		1996	I	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$\bar{1}\ \underline{2}\ 3_2\ 4\ \underline{5}_2$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$\bar{\bar{2}}\ 4_2\ 6_1\underline{6}_2\bar{6}_3\ \bar{\bar{7}}_1\ 8_2\ \underline{9}_2$
			II	$\bar{2}\ 4\ \underline{6}_1\ 7_1\bar{7}_2$	$1\ \underline{2}\ 3_2\ 4\ 5_1$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{4}$	$2\ 4_2\ 6_1\underline{6}_2\bar{6}_3\ \bar{7}_1\ 8_2\ \underline{9}_2$
TRI 1648	16	1948	I	$6_1\underline{6}_2\ \underline{7}_1\bar{7}_2$	$\underline{2}\ 3_2\ \underline{4}\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$3\ 4_2\ 6_1\underline{6}_3\ 8_2\ \underline{9}_2$
		1983	I	$6_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2 3_3\ \underline{4}\ 5_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$3\ 6_1\underline{6}_3\ 8_2\ \underline{9}_2$
TRI 3342	16	1951	I	$3\ 5\ 6_1\ \underline{7}_1\bar{7}_2$	$1\ \underline{2}_1\ 3_2\ \underline{4}\ \bar{5}_2$	$2_1 2_3\ \underline{3}\ 4$	$\underline{4}_1\bar{\bar{4}}_3\ \bar{\bar{6}}_1\ 7_1\ 8_2\ \underline{9}_2$
		1995	I	$3\ 5\ 6_1\ \underline{7}_1\bar{7}_2$	$1\ \underline{2}_1\ 3_2\ \underline{4}\ \bar{5}_2$	$2_1 2_3\ \underline{3}\ 4$	$\underline{4}_1\bar{\bar{4}}_3\ \bar{6}_1\ 7_1\ 8_2\ \underline{9}_2$
TRI 1646	16	1948	I	$\underline{6}_1\ \underline{7}_1\bar{7}_2$	$\underline{2}\ 3_2 3_3\ \underline{4}\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$3\ 4_2\ \underline{6}_3\ \underline{8}_2\ \underline{9}_2$
		1979	I	$\underline{6}_1\ \underline{7}_1\bar{7}_2$	$\underline{2}\ 3_2 3_3\ \underline{4}\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$3\ 4_2\ \underline{6}_3\ \underline{8}_2\ \underline{9}_2$
			II	$\underline{6}_1\ \underline{7}_1$	$\underline{2}\ 3_2\ \underline{4}\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{4}$	$3\ 4_2\ \underline{6}_3\ 7_1\ \underline{8}_2\ \underline{9}_2\ \bar{10}_1$
TRI 1634	17	1948	I	$\underline{6}_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2 3_3\ 4\ 5_2$	$2_1 2_3\ \underline{3}\ \underline{5}$	$\underline{4}_1\underline{4}_3\ 5\ 6_1\ \underline{7}_1\ 8_2\ 9_2\ \bar{10}_1$
		1996	I	$\underline{6}_1\ \underline{7}_1 7_2$	$\underline{2}\ 3_2 3_3\ \underline{4}\ \underline{5}_2$	$\bar{2}_1 2_3\ \underline{3}\ \underline{5}$	$\underline{4}_1\underline{4}_3\ 5\ 6_1\ \underline{7}_1\ 8_2\ 9_2\ \bar{10}_1$
TRI 2519	24	1948	I	$\underline{6}_1\ \underline{7}_1\underline{7}_2$	$\underline{2}\ 3_2\ \underline{4}\ \underline{5}_1$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{5}$	$\underline{4}_1 4_3\ 6_1\ \bar{7}_1\ \underline{8}_2\ \underline{9}_2$
			II	$\underline{6}_1\ \underline{7}_1\underline{7}_2$	$\underline{2}\ 3_2\ \underline{4}\ \bar{5}_2$	$1\ \bar{2}_1 2_3\ \underline{3}\ 4$	$\underline{4}_1\underline{4}_3\ \bar{6}_1\ \bar{7}_1\ 8_1\ 8_2\ 9_2$
		1996	I	$\underline{6}_1\ \underline{7}_1\underline{7}_2$	$\underline{2}\ 3_2\ \underline{4}\ \underline{5}_1$	$\bar{2}_1\bar{2}_3\ \underline{3}\ \underline{5}$	$\underline{4}_1\underline{4}_3\ 6_1\ \bar{7}_1\ \underline{8}_2\ \underline{9}_2$
			II	$\underline{6}_1\ \underline{7}_1\underline{7}_2$	$\underline{2}\ 3_2\ \underline{4}\ 5_1\bar{5}_2$	$2_1\bar{2}_3\ \underline{3}\ \underline{5}$	$\underline{4}_1\underline{4}_3\ 6_1\ 7_1\ \underline{8}_2\ \underline{9}_2$

1-5-9 – positions of protein components in electrophoretic patterns in accordance with etalon (standard) pattern; 6_2 – subposition '2' of component '6'; $\underline{6}$ – intensive component; $\bar{\bar{6}}$, $\bar{6}$ – very weak and weak intensity of components, respectively.

different kinds of plants. A large number of such researches was made by using isozymes and storage proteins (Konarev et al. 1995, 1996; Allard 1997). More recently, DNA-marker techniques in general but microsatellite markers in particular were applied for these purposes (Weising et al. 1995; Kresovich et al. 1997; Börner et al. 2000; Chebotar et al. 2003). The material of the present investigation was previously studied by using SSR-markers (Börner et al. 2000). In some cases, it was not possible to receive DNA amplification for single seeds of early reproductions, which had been stored at room temperature.

For the accession TRI 4599, the authors found two alleles for three out of nine SSR-markers in the original sample of which only one remained after 15 regenerations, due to genetic drift. This was confirmed in the present study analysing single seed gliadin spectra of the samples. Pattern of type III identified in accession TRI 4599 in 1952 with a frequency of about 15% was not detected in the seed sample harvested in 1996.

Deviating results were obtained for accessions TRI 249 and TRI 1646. In the sample of the last reproduction of TRI 249 genotypes with pattern type III were discovered, which were absent in the sample of the first reproduction. For control purpose we investigated 10 additional grains from the original sample, however, grains with the type III spectrum did not appear. It should be mentioned that the seeds of the first regeneration maintained in the herbarium collection are limited and we could not increase the number further. It may be possible, that the genotypes with pattern type III were present in the original sample, but with a very low frequency of occurrence, which may have increased during 11 reproductions.

A similar picture was found for TRI 1646. Here only one pattern was detected in the original sample, whereas in the seeds of the latest reproduction a minor genotype of pattern type II appeared. Using the SSR-marker system no differences between early and late reproductions of accession TRI 249 and TRI 1646 were detected. The latter, however, was re-tested with one marker only.

The present research confirmed conclusions of Konarev et al. (1995, 1996), Steiner et al. (1997), Kresovich et al. (1997), Hodgkin (1997) or Börner et al. (2000) about the necessity to control the genotypic structure or genetic integrity of accessions reproduced in genebanks. In fact, genebank pioneers as Vavilov, Mansfeld or Lehmann outlined the requirement for the description of accessions in genebank collections and suggested the comparison with reference material for monitoring the status of genebank accessions after regeneration already decades earlier (Mansfeld 1951; Lehmann and Mansfeld 1957). Morphological traits were used for the development of classification keys. However, only characters having a high genetic content give consistent and correct classifications (Lehmann and Blixt 1984).

The efficiency of using single seed storage protein spectra for studying the genotypic structure of genebank collections was demonstrated by Schulze et al. (1994), Konarev et al. (1996, 2002) or Steiner et al. (1997). The possibility to investigate old and even dead seeds by single seed analysis allows the estimation of the dynamics of the genotypic composition of an accession after many years and provides a large resolution ability in detection of genetic drift in populations. The large resolution ability of the gliadin (prolamin) system is conditioned by the high number of allele variants at gliadin loci (Vvedenskaya et al. 1993; Konarev et al. 1995, 1996, 2002; Weising et al. 1995). Molecular marker systems (proteins, isozymes, DNA markers) are powerful tools for controlling the genetic integrity of genebank collections and the dynamics of populations.

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