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GENETIC INTEGRITY OF *EX SITU* GENEBANK COLLECTIONS

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Abstract: The genetic integrity of four accessions of the cross-pollinating species rye (*Secale cereale* L.) was investigated. Seeds available from the first and most recent regeneration cycles, multiplied 8, 12 (twice) or 14 times were fingerprinted using microsatellite markers. In all four accessions the allele numbers and frequencies changed after regeneration. Alleles present in the original seed sample were not detectable in the regenerated populations, whereas on the other hand, alleles were found in the recent seed sample, which were not observed in the investigated plants of the original one.

Key Words: Cross-Pollinating Species, *Ex situ* Maintenance, Fingerprinting, Genebank Management, Genetic Integrity, Molecular Markers, Rye, *Secale cereale* L.

INTRODUCTION

In the Gatersleben genebank about 100,000 accessions are maintained including cereals, legumes, vegetables, oil and fibre plants and medicinal herbs. Depending on the storage conditions and the frequency of providing genebank material to users regeneration becomes necessary. For that different procedures have to be applied regarding to the pollination systems of the particular crops. Especially cross-pollinating species need extended efforts in order to maintain the genetic integrity of the germplasm accessions. However, a contamination by foreign pollen or incorrect handling during multiplication may affect the genetic integrity of self-pollinating species as well. Randomly selected accessions from the Gatersleben genebank collection belonging to the self-pollinating species *Triticum aestivum* L. were investigated by employing molecular markers [1]. The frequencies of multiplication varied between 5 and 24. The analyses of the stocks showed a high degree of identity. No contamination due to foreign pollen or incorrect handling during the multiplication cycles was discovered.

Previous studies of variability and relationships among populations of cross pollinating species like rye (*Secale cereale* L.) most concentrated on using isozyme markers [2-5] or RAPD and ISSR markers [6, 7]. Another powerful technique for detecting DNA polymorphism is the microsatellite analysis. This analysis makes it possible to determine the genotypes of individuals and is very useful for detecting population bottlenecks, inbreeding and other genetic parameters of populations [8, 9].

In order to get some information about the integrity of cross-pollinating species maintained in *ex situ* seed banks, and especially about *Secale cereale* L. four randomly selected accessions of rye originated from Austria (Acc1), Germany (Acc2, Acc3) and Italy (Acc4) were studied by using microsatellite markers. The obtained results will be used for monitoring, controlling and improving the efficiency of genebank maintenance of rye.

MATERIAL AND METHODS

Seeds from the first regeneration performed more than 35 years ago and stored as reference collection were compared with those obtained from the most recent multiplication saved in the cold seed store. The material was regenerated 8 (Acc1), 12 (Acc2, Acc3) or 14 (Acc4) times. Because the rye accessions represent populations, 36 single grains from both the first and most recent regeneration cycle of each accession were used for extracting DNA. Successfully analysed were 26 to 36 samples per microsatellite/accession combination. Seven rye microsatellite markers (RMS) designated RMS10, RMS12, RMS18, RMS28, RMS104, RMS115 and RMS121 were chosen for analysis. PCR reactions and fragments detection were performed as described for wheat [10, 11].

RESULTS AND DISCUSSION

For all four accessions it was found that the frequencies and/or the numbers of the alleles changed after 8 to 14 regeneration cycles. In 25 of the 28 analysed accession/marker combinations less alleles (nearly 50 %) were discovered whereas in 16 cases alleles were found in the populations regenerated recently, but not in the investigated plants of the original one (Table 1). Examples obtained with the marker RMS12 are given in Figure 1. For Acc1 alleles having 152, 154, 158, 192, 198, 202, 215 and 218 base pairs could be detected in the seeds originated from the herbarium collection only. On the other hand alleles having 180, 186 and 208 base pairs, the allele 186 base pairs with a quite high frequency were found only in the population available in the cold seed store. Analysing Acc 2 again alleles present in the sample taken from the

Tab. 1. Number of alleles detected in the seed samples taken from the first and most recently grown regeneration plots.

Accession	Progenies after 1st multiplication	Years with winter damage	rms10				rms12				rms18				rms28				rms 104				rms107				rms115				rms121			
			A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Acc 1; 1963 & 1998	7	1971***,1979***	4	3	1	0	12	4	8	3	13	4	9	1	6	3	3	2	13	9	4	0					10	3	7	2	10	7	3	1
Acc 2; 1954 & 1993	11	1958 *,1962 ***	3	3	0	0	15	6	9	0	10	2	8	2	9	6	3	0	12	5	7	4	8	4	4	3	11	7	4	1	12	9	3	0
Acc 3; 1954 & 1995	11	1957 **,1972 *, 1976*,1977*	3	3	0	0	10	4	6	0	8	4	4	1	6	3	3	0	6	4	2	0	3	2	1	0	7	1	6	1	10	3	7	0
Acc 4; 1953 & 1995	13	1957 ***,1976 *	1	1	0	2	14	4	10	4					9	2	7	1	8	5	3	1					9	3	6	2				

*about 50% of plants lost, ** about 75% of plants lost, *** about 90% of plants lost

A: Number of alleles present in the sample taken from the first regeneration,
B: Number of alleles common to A and still present in the sample taken from the most recent regeneration,
C: Number of alleles not detected in the sample taken from the most recent regeneration,
D: Number of alleles different to A present in the sample taken from the most recently grown multiplication plots

first regeneration cycle could not be detected in the progeny obtained from the most recent regeneration. New alleles were not detected.

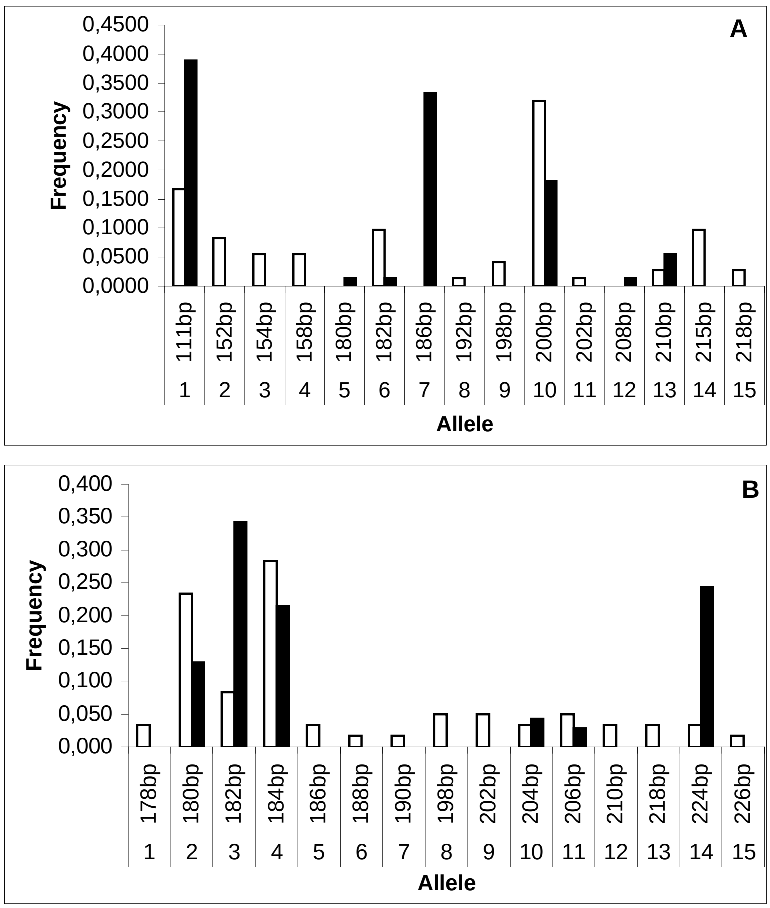

Fig. 1. Allele frequencies detected for marker RMS12 analysing seed samples of the accessions Acc1 (A) and Acc2 (B) taken from the first (white columns) and most recently (black columns) grown regeneration plots.

It is clearly demonstrated that in the four, randomly selected rye accessions both the frequency and the number of alleles was changed as a consequence of regenerating them (Table 2). By using microsatellite markers we found, that in case of a reduction of the size of an accession, representing a population, genetic drift was enhanced and, therefore, alleles got possibly lost. Reasons for

Tab. 2. Allele frequency of microsatellite loci in sub-populations of rye accessions

No	Locus	Allele size (bp)	Allele frequency							
			Acc 1, 1963	Acc 1, 1998	Acc 2, 1954	Acc 2, 1993	Acc 3, 1954	Acc 3, 1995	Acc 4, 1953	Acc 4, 1995
	RMS 10		n=32	n=34	n=28	n=34	n=36	n=36	n=31	n=35
1		213	0,328		0,161	0,221	0,167	0,014		0,271
2		215	0,422	0,926	0,250	0,750	0,292	0,431		0,086
3		217	0,234	0,059	0,589	0,029	0,542	0,556	1,000	0,643
4		224	0,016	0,015						
	RMS 12		n=36	n=36	n=30	n=35	n=36	n=36	n=33	n=36
5		111	0,167	0,389			0,097		0,152	
6		152	0,083				0,056	0,264	0,045	
7		154	0,056						0,091	
8		158	0,056						0,061	0,403
9		178			0,033					0,347
10		180		0,014	0,233	0,129	0,056		0,030	0,083
11		182	0,097	0,014	0,083	0,343	0,264	0,069		
12		184			0,283	0,214				
13		186		0,333	0,033		0,014		0,045	
14		188			0,017				0,030	
15		190			0,017					0,042
16		192	0,014						0,030	0,056
17		195							0,076	
18		198	0,042		0,050					
19		200	0,319	0,181						
20		202	0,014		0,050				0,258	
21		204			0,033	0,043				
22		206			0,050	0,029	0,014			
23		208		0,014			0,028		0,076	
24		210	0,028	0,056	0,033		0,111	0,194	0,045	0,014
25		212					0,264	0,472		
26		215	0,097							0,014
27		218	0,028		0,033					0,042
28		220					0,097			
29		224			0,033	0,243			0,030	
30		226			0,017				0,030	
	RMS 18		n=36	n=35	n=36	n=36	n=36	n=36		
31		123	0,056	0,014	0,083					
32		128		0,243						
33		132	0,014							

Tab. 2. (continued)

No	Locus	Allele size (bp)	Allele frequency							
			Acc 1, 1963	Acc 1, 1998	Acc 2, 1954	Acc 2, 1993	Acc 3, 1954	Acc 3, 1995	Acc 4, 1953	Acc 4, 1995
34		134	0,028	0,157			0,014			
35		136	0,111		0,139	0,319	0,292	0,236		
36		138	0,028		0,014			0,125		
37		140	0,181		0,056	0,514	0,194			
38		142			0,014					
39		144	0,111		0,125		0,056	0,056		
40		146	0,028	0,543	0,236		0,194	0,431		
41		148	0,222		0,167		0,028			
42		150	0,056		0,056					
43		152	0,097	0,043	0,111					
44		154				0,083	0,083			
45		156				0,083				
46		162	0,056							
47		168					0,139	0,153		
48		178	0,014							
	RMS 28		n=26	n=36	n=36	n=28	n=36	n=36	n=32	n=36
49		234							0,016	
50		236							0,016	
51		238			0,125		0,444	0,250		
52		240	0,308	0,139	0,444	0,482	0,278	0,736	0,375	0,819
53		242			0,083	0,071			0,094	
54		244	0,038	0,278	0,042		0,042		0,219	
55		246	0,577	0,236	0,153	0,161	0,194		0,094	0,167
56		248	0,038		0,056	0,161			0,016	
57		250	0,019		0,028	0,071	0,028	0,014		0,014
58		252			0,056	0,054			0,063	
59		254		0,111	0,014		0,014		0,109	
60		256		0,236						
61		270	0,019							
	RMS 104		n=34	n=35	n=36	n=32	n=36	n=36	n=32	n=36
62		null	0,059	0,029						
63		136				0,016				
64		143	0,044							0,056
65		145				0,172				
66		147	0,118	0,071	0,056	0,047			0,063	

Tab. 2. (continued)

No	Locus	Allele size (bp)	Allele frequency							
			Acc 1, 1963	Acc 1, 1998	Acc 2, 1954	Acc 2, 1993	Acc 3, 1954	Acc 3, 1995	Acc 4, 1953	Acc 4, 1995
67		149	0,103	0,129			0,236	0,306	0,438	
68		151			0,014					
69		153	0,015	0,114	0,028	0,078	0,194	0,264	0,250	0,069
70		155	0,044	0,186	0,042		0,028	0,097	0,063	0,042
71		157	0,206	0,100	0,139	0,203	0,319	0,333	0,047	
72		159	0,191	0,086	0,458	0,422	0,153		0,016	0,458
73		161	0,015	0,029	0,097		0,069		0,078	0,250
74		163	0,015		0,042	0,016			0,047	0,125
75		165	0,103			0,047				
76		167	0,044		0,014					
77		169	0,044	0,257	0,069					
78		171			0,014					
79		177			0,028					
	RMS 107				n=36	n=36	n=34	n=32		
80		null			0,028	0,028				
81		79				0,056				
82		81			0,625	0,403	0,632	0,516		
83		83				0,028				
84		103			0,111	0,250	0,059			
85		105			0,056		0,309	0,484		
86		107			0,111	0,153				
87		109				0,083				
88		111			0,014					
89		119			0,028					
90		125			0,028					
	RMS 115		n=32	n=33	n=34	n=33	n=35	n=34	n=32	n=36
91		110			0,029	0,030				
92		112			0,044	0,136			0,063	
93		114		0,364	0,426	0,591			0,063	
94		116	0,063	0,227	0,029	0,076			0,266	0,889
95		118		0,152	0,029	0,045			0,031	0,056
96		120	0,094		0,176	0,000	0,086		0,250	
97		122	0,094	0,167	0,103	0,015	0,171		0,078	0,028
98		124	0,094				0,171		0,141	

Tab. 2. (continued)

No	Locus	Allele size (bp)	Allele frequency							
			Acc 1, 1963	Acc 1, 1998	Acc 2, 1954	Acc 2, 1993	Acc 3, 1954	Acc 3, 1995	Acc 4, 1953	Acc 4, 1995
99		126	0,156		0,088	0,030		0,191		
100		128	0,063	0,091			0,014		0,078	
101		130	0,219			0,030	0,243	0,809	0,031	
102		132			0,029		0,186			
103		134	0,047		0,015		0,129			
104		138			0,029					
105		142	0,063							
106		146								0,014
107		160	0,109			0,045				
108		174								0,014
	RMS 121		n=28	n=36	n=36	n=36	n=36	n=36		
109		154	0,054	0,014	0,153		0,125			
110		164		0,028						
111		168			0,028					
112		170	0,143	0,194	0,278	0,111	0,083			
113		172	0,232	0,097	0,014	0,028	0,014			
114		174	0,179		0,153	0,097				
115		176	0,036	0,347	0,014	0,069	0,153	0,153		
116		178	0,036	0,083	0,083	0,181				
117		180	0,107	0,167	0,097	0,194	0,278	0,694		
118		182	0,107	0,069	0,028	0,167	0,042			
119		184	0,036							
120		186					0,014			
121		188	0,071		0,014	0,014	0,056	0,153		
122		192			0,097		0,097			
123		194			0,042	0,139	0,139			

loosing alleles may be that the population sizes used for regeneration of the material were too small or decreased by damage during periods of environmental stress. The mutation rate at microsatellite loci is considered to be 10^{-4}~10^{-5} per generation in mouse [12], 10^{-4} in human and 10^{-6} in Drosophila [13]. So the changes that were revealed are most liking not due to mutation events at microsatellite loci or primer annealing sites. New alleles may be due to contamination with foreign pollen. Although only four accessions were investigated so far it may be suggested that genetic changes occur in other rye accessions as well and, most probably also in accessions of other cross-pollinating species maintained in *ex situ* genebanks.

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