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A comparative assessment of genetic diversity in cultivated barley collected in different decades of the last century in Austria, Albania and India by using genomic and genic simple sequence repeat (SSR) markers

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Abstract

Molecular investigations of qualitative and quantitative changes in the genetic diversity of cultivated crops are useful for plant breeding and the management of crop genetic resources. A genotyping study, based on 28 genomic (g-SSR) and 13 expressed sequence tag-derived (e-SSR) microsatellite markers uniformly distributed across the barley genome, was conducted on samples of cultivated barley (*Hordeum vulgare* L.) collected at intervals of 40–50 years in three comparable geographical regions in Austria, Albania and India. The analysis indicated an absence of any significant differences either in the total number of alleles per locus or in g-SSR and e-SSR polymorphic information content (PIC) values from the Indian and Austrian materials. However, a slight reduction in genetic diversity was noted among the materials collected in Albania. The trend in numbers of collection mission-specific SSR alleles suggests significant allele flow over the time interval sampled. The g-SSRs yielded a higher mean number of alleles per locus and a superior PIC than the e-SSR markers. We conclude that a qualitative, rather than a quantitative shift in diversity has taken place over time, and that g-SSR markers detect more diversity than do e-SSR markers.

Keywords: barley; e-SSRs; genebank collections; genetic diversity; g-SSRs; *Hordeum vulgare* L.; microsatellites

Introduction

Allelic diversity in germplasm collections is of central interest to plant breeding and crop improvement programmes. However, urbanization, the replacement of traditional agricultural systems by modern industrial methods and the introduction of modern high-yielding varieties all present a threat to biological diversity. The *ex situ* conservation of plant genetic resources plays an important role in the conservation of both intra- and inter-specific diversity of both crop species and their wild relatives. The Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) genebank presently holds about 150,000 accessions of various plant species, including 21,000 of barley (*Hordeum vulgare* L.) (IPK 2004 Annual Report). To create this collection, more that 140 expeditions have been made to different parts of the world, starting in the 1920s. Specifically for

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cereal landraces, E. Mayr collected in the Austrian Alps from 1922 to 1932, A. Herrlich and collaborators collected in the Himalayas (India, Nepal) in the 1930s, while H. Stubbe and his associates collected in the Balkans (Albania, Greece) in the early 1940s (for references see Khlestkina *et al.*, 2004a). Several decades later, some of these collection missions were repeated, although not always in exactly the same areas as earlier. These 'repeat missions' included Nepal (1971), Northern India (1976), Austria (1982, 1983, 1986) and Albania (1993, 1994). Thus the germplasm collections available in the IPK genebank provide an opportunity to investigate changes in genetic diversity in materials collected from comparable geographic regions on different expeditions (Khlestkina *et al.*, 2004a).

A variety of molecular markers are available for the assessment of diversity in germplasm collections (see Varshney *et al.*, 2004). Due to their co-dominance and multi-allelism, microsatellites or simple sequence repeats (SSRs) have proven to be the markers of choice for diversity studies (Gupta and Varshney, 2000; Röder *et al.*, 2004). In addition to SSR markers derived from genomic DNA (g-SSRs), SSR assays have also been developed from expressed sequence tag (EST) or genic sequences (e-SSRs), and the latter have been recommended for the assessment of functional diversity in natural populations and germplasm collections (see Varshney *et al.*, 2005). In the present study we have used a set of both g-SSRs and e-SSRs, uniformly distributed across all the barley chromosomes, to genotype a selection of barley accessions from the IPK genebank. The materials originate from the successive expeditions to Austria, Albania and Northern India, with the following objectives: (i) to assess quantitative as well as qualitative differences in genetic diversity in germplasm collections, and (ii) to compare the potential of g-SSR and e-SSR markers for the assessment of genetic diversity.

Material and methods

Plant material and SSR analysis

The chosen set of 96 barley accessions was comprised of 18 accessions per collection mission for the Albanian and Indian material, and 12 per collection mission for the Austrian material (Table 1). The geographical distribution of the collection sites within each country was comparable (Fig. 1). Total genomic DNA was extracted from pools of five grains per accession, according to the procedure described by Plaschke *et al.* (1995). A set of 28 g-SSRs and 13 e-SSRs, genetically distributed across all seven barley linkage groups, was chosen for the analysis. The primer sequences and genetic locations of the

Table 1. Barley accessions from successive collection missions performed in Austria, Albania and India

Country of origin	Collection mission	Accessions investigated
Austria	1922–1932	HOR 3949, HOR 3950, HOR 3951, HOR 3952, HOR 3953, HOR 3954, HOR 3955, HOR 3956, HOR 3957, HOR 3958, HOR 3959, HOR 3960
	1982	HOR 9848, HOR 9850, HOR 9851, HOR 9852, HOR 9853, HOR 9854, HOR 9855, HOR 9856, HOR 9857, HOR 9858, HOR 9859, HOR 10094
Albania	1941	HOR 736, HOR 739, HOR 740, HOR 741, HOR 750, HOR 754, HOR 755, HOR 756, HOR 770, HOR 772, HOR 938, HOR 939, HOR 964, HOR 1269, HOR 1281, HOR 1287, HOR 1381, HOR 1388
	1994	HOR 11312, HOR 11313, HOR 11314, HOR 11315, HOR 11316, HOR 12397, HOR 12398, HOR 12399, HOR 12400, HOR 12401, HOR 12402, HOR 12403, HOR 12413, HOR 12414, HOR 12415, HOR 12416, HOR 12798, HOR 12799
India	1937	HOR 150, HOR 152, HOR 154, HOR 1271, HOR 1273, HOR 1348, HOR 1451, HOR 1552, HOR 1577, HOR 1579, HOR 1581, HOR 1657, HOR 1655, HOR 1658, HOR 1684, HOR 1765, HOR 1799, HOR 2389
	1976	HOR 8316, HOR 8318, HOR 8324, HOR 8355, HOR 8365, HOR 8372, HOR 8373, HOR 8378, HOR 8383, HOR 8384, HOR 8393, HOR 8403, HOR 8409, HOR 8414, HOR 8430, HOR 8438, HOR 8444, HOR 8451

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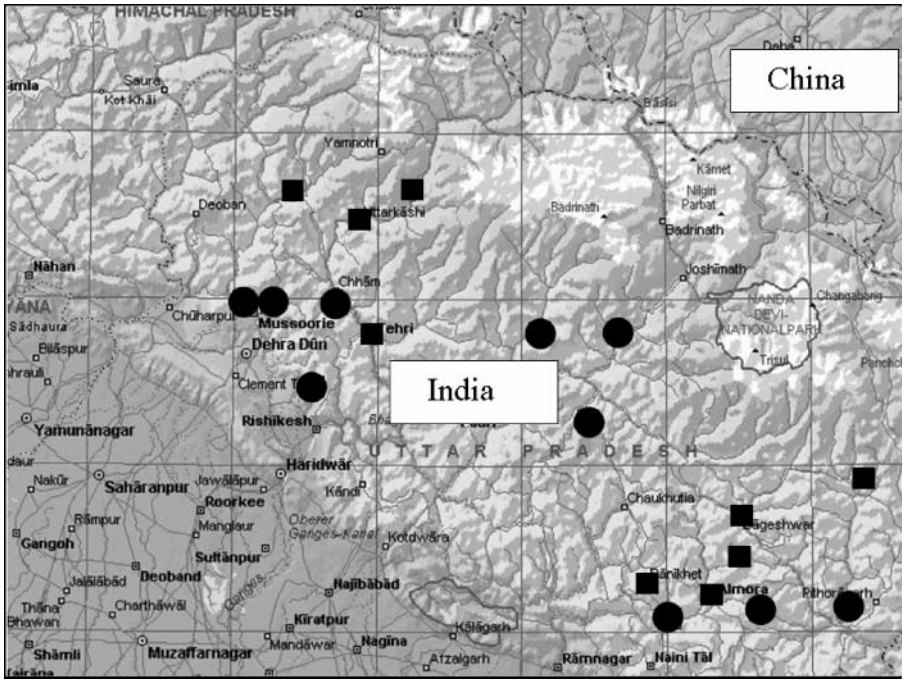

Fig. 1. Collection sites of successive missions carried out in India in 1937 (■) and 1976 (●).

g-SSR loci were as described by Liu *et al.* (1996), Ramsay *et al.* (2000) and Li *et al.* (2003). PCR and fragment detection were performed according to Röder *et al.* (1998). In order to ensure size accuracy, the cultivars 'Brenda' and 'Morex' were used as controls in each run. Primer sequences and map positions of four of the e-SSRs (GBM1002, GBM1007, GBM1020 and GBM1059) have been previously described by Thiel *et al.* (2003), and those of the remaining nine e-SSRs are available on request. Amplification of microsatellite loci using fluorescence dye-labelled primer pairs was carried out as described by Thiel *et al.* (2003). Amplification products were separated on an ABI377 fragment analyser and evaluated using GenoTyper 3.7 (Applied Biosystems). In order to ensure size accuracy, the cultivar 'Barke' was used as control in each run, as e-SSR markers were derived from ESTs generated from various tissues of this cultivar.

Data analysis

Numbers of collection mission-specific and shared alleles were counted for each microsatellite locus. Allelic polymorphic information content (PIC) was calculated as $PIC = 1 - \Sigma(Pi)^2$, as suggested by Anderson *et al.* (1993) (*Pi* is the proportion of the population carrying the *i*th allele calculated for each locus). Collection mission means were compared using the Mann and Whitney (1947) parameter-free test (*U*-test).

Results

Quantitative differences in genetic diversity

g-SSR loci

The 28 g-SSR loci generated 174 (mean 6.2 per locus), 221 (7.9 per locus) and 256 (9.1 per locus) alleles in, respectively, the Austrian, Albanian and Indian accessions. With respect to mission-specific alleles, 44 (1.6 per locus) and 34 (1.2 per locus) alleles were found in, respectively, the first and later collection missions to Austria. Likewise, for the Albanian materials, there were 88 (3.1 per locus) and 30 (1.1 per locus) mission-specific alleles, and 69 (2.5 per locus) and 72 (2.6 per locus) in the Indian materials. No significant differences were detected in the total number of alleles per locus within the material of the successive collection missions in Austria and India (in all cases, $P \geq 0.05$, two-tailed *U*-tests). However, for the Albanian material, there was a significant difference ($P < 0.001$, two-tailed test) in allele number between the 1941 and 1994 collections. In this latter case, the total number of alleles per locus decreased by 30% during the 53-year period (Fig. 2a). A similar trend was observed for the mean PIC values (Table 2). The *U*-test did not reveal any significant difference in the mean PIC values between collection periods in either Austria or India (in all cases, $P \geq 0.05$, two-tailed tests), whereas the PIC values for the Albanian materials were 0.72 for the 1941 collection and 0.62 for the 1994

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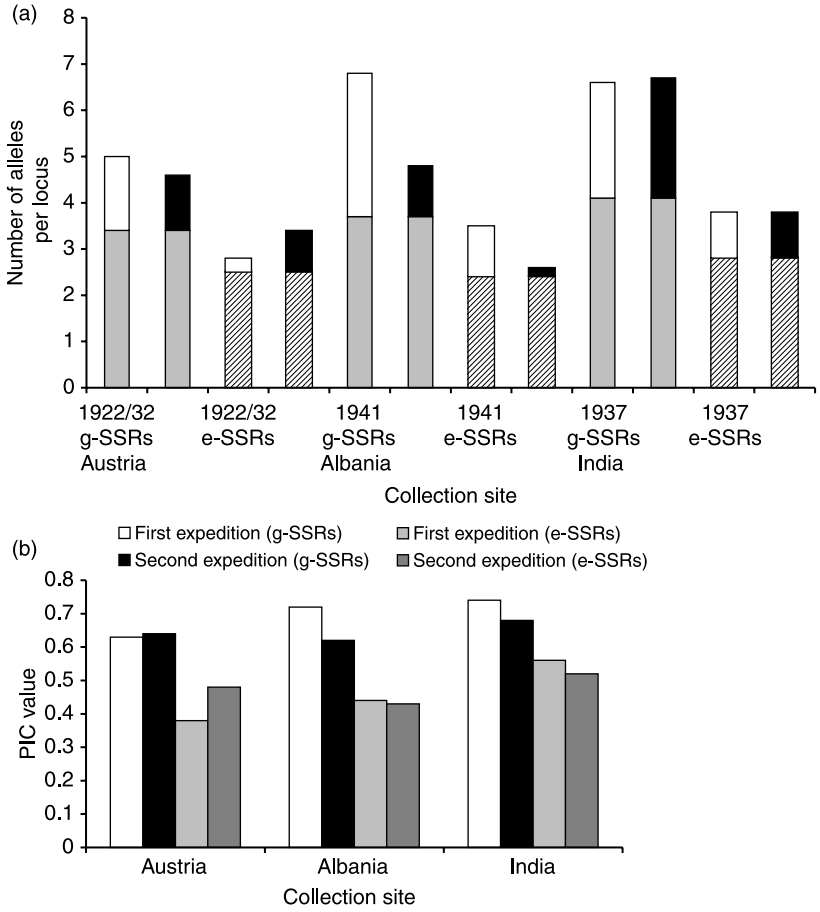

Fig. 2. A comparison of the mean number of alleles per locus (a) and average PIC value (b) detected in barley accessions collected during two expeditions in Austria (1922/32 and 1982), Albania (1941 and 1994) and India (1937 and 1976), on the basis of g-SSR and e-SSR genotyping. (a) Collection mission-specific alleles are shown as white (first expedition) and black (successive expedition) columns, while common alleles are indicated in grey (g-SSR) and shaded (e-SSR) columns.

collection, and this difference was statistically significant ($P < 0.01$, two-tailed test) (Fig. 2b).

e-SSR loci

The 13 e-SSR loci generated 49 (3.8 per locus), 48 (3.7 per locus) and 63 (4.8 per locus) alleles in the three collections (Table 3). Comparing the different collection missions, there were 5 (0.4 per locus) and 12 (0.9 per locus) alleles for the Austrian materials, 14 (1.1 per locus) and 3 (0.2 per locus) for the Albanian materials, and 13 (1.0 per locus) and 14 (1.1 per locus) for the Indian material. None of these differences in allele number (Table 3) or PIC value (Table 4) for the individual collection missions were statistically significant (in all cases $P \geq 0.05$, two-tailed tests) (Fig. 2).

Qualitative differences in genetic diversity

A major proportion of the g-SSR alleles was shared between materials from both collection periods in Austria (55%), Albania (47%) and India (45%), whereas the remainder of the alleles were collection year-specific, indicating a significant qualitative difference in barley genetic diversity during the 40–60 years of the last century in all the geographical regions studied (Table 5; Fig. 2a). Similarly, over half of all alleles (65% in Austria and Albania and 58% in India) detected by the 13 e-SSR markers were shared between two collection missions across all three countries. In all, about 40% of alleles were collection mission-specific across all three geographic regions (Table 3; Fig. 2a).

Comparison between g-SSR and e-SSR markers

In addition to assessing diversity changes, the present study allows a comparison of the effectiveness of g-SSR and e-SSR markers for genetic diversity studies (Fig. 2). The range in allele number per locus detected by the two marker types was, respectively, 6.2–9.1 and 3.7–4.8. Similarly, the mean PIC values lay in the ranges

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Table 2. Diversity index (PIC value) at g-SSR loci

		Geographical region					
		Austria		Albania		India	
Chromosome and marker		1922–1932	1982	1941	1994	1937	1976
1H	GBMS0184	0.54	0.72	0.76	0.73	0.67	0.57
	Bmag0211	0.60	0.53	0.68	0.53	0.84	0.85
	Bmag0718	0.73	0.75	0.78	0.73	0.78	0.62
	Bmac0032	0.74	0.73	0.89	0.78	0.89	0.85
2H	Bmag0518	0.77	0.68	0.72	0.69	0.87	0.87
	GBMS0247	0.68	0.56	0.72	0.60	0.79	0.86
	GBMS0160	0.42	0.41	0.64	0.59	0.74	0.74
	HVM0036	0.69	0.71	0.73	0.69	0.50	0.71
3H	Bmag0013	0.73	0.78	0.88	0.67	0.80	0.88
	Bmag0225	0.84	0.77	0.87	0.78	0.76	0.77
	GBMS0189	0.85	0.80	0.81	0.75	0.75	0.81
	Bmag0603	0.42	0.65	0.79	0.77	0.84	0.79
4H	GBMS0133	0.49	0.54	0.76	0.32	0.69	0.61
	EBmac0701	0.78	0.77	0.84	0.60	0.83	0.66
	GBMS0087	0.39	0.14	0.53	0.39	0.77	0.47
	EBmac0788	0.80	0.74	0.82	0.53	0.83	0.69
5H	GBMS0032	0.73	0.67	0.74	0.61	0.58	0.28
	EBmac0684	0.46	0.70	0.47	0.62	0.41	0.49
	CBMS0119	0.46	0.60	0.62	0.63	0.69	0.69
	GMS0001	0.54	0.50	0.68	0.41	0.64	0.56
6H	Bmac0316	0.72	0.54	0.69	0.65	0.70	0.64
	Bmac0040	0.78	0.82	0.90	0.86	0.87	0.82
	EBmac0602	0.00	0.29	0.46	0.24	0.85	0.76
	Bmag0613	0.77	0.73	0.42	0.76	0.90	0.72
7H	GBMS0192	0.55	0.61	0.79	0.73	0.50	0.54
	GBMS0035	0.56	0.66	0.57	0.35	0.73	0.54
	EBmac0755	0.79	0.70	0.85	0.68	0.76	0.71
	Bmag0135	0.79	0.83	0.79	0.75	0.78	0.60
Mean		0.63	0.64	0.72	0.62	0.74	0.68

0.62–0.74 and 0.38–0.56, respectively. Although the g-SSRs yield a higher number of alleles and display a higher PIC value than the e-SSRs, both the trend in allele number and in PIC value was comparable between materials collected in the two missions, with the exception of Austria (Fig. 2). In the latter case, both allele number and PIC value were higher for e-SSRs in the later mission, whereas allele number was smaller, despite a slight rise in PIC value, for the g-SSRs (Tables 2–5; Fig. 2). The differences, however, were not statistically significant ($P \geq 0.05$, two-tailed tests).

Discussion

Because of the many advantages over other classes of molecular markers, microsatellite markers have been extensively used for genetic diversity studies in a range of plant species, including barley (Saghai Maroof *et al.*, 1994; Becker and Heun, 1995; Struss and Plieske, 1998). Recently available e-SSR markers have also been utilized for genetic diversity studies in barley (Pillen *et al.*, 2000; Kota *et al.*, 2001; Thiel *et al.*, 2003; Russell *et al.*, 2004). Therefore we employed both types of markers to detect differences in genetic diversity over the two time periods and three collection areas.

Genetic diversity in barley

No significant differences were observed in allele number per locus or PIC in both missions in Austria and India, whether using g-SSR or e-SSR markers. However, for the g-SSRs, a loss of 30% of the number of alleles per locus was noted in the later mission in Albania. Similarly, a significant reduction of 0.10 (15%) PIC was found for g-SSRs. For the e-SSRs the loss was in the order of 25%, although this difference was not statistically significant. Interestingly, a similar tendency was observed in an earlier genetic diversity study among Albanian wheat accessions (Khlestkina *et al.*, 2004a), where although the difference in mean allele number was not statistically significant between

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Table 3. Chromosomal location and numbers of collection mission-specific and shared alleles for e-SSR loci

Chromosome and marker		Geographical region								
		Austria			Albania			India		
		No. of specific alleles			No. of specific alleles			No. of specific alleles		
		1922–1932	1982	No. of shared alleles	1941	1994	No. of shared alleles	1937	1976	No. of shared alleles
1H	GBM1002	0	0	2	0	0	2	1	0	1
	GBM1007	1	2	4	1	0	4	2	3	2
	GBM1020	0	0	2	0	0	2	0	0	2
2H	GBM1059	0	1	4	2	0	3	0	1	6
	GBM1208	0	2	1	0	1	1	2	0	3
3H	GBM1218	1	2	1	1	0	4	1	3	1
	GBM1221	1	1	2	2	1	1	0	0	5
	GBM1280	1	0	3	1	0	2	0	0	5
4H	GBM1404	0	0	1	0	0	2	0	1	1
	GBM1419	0	0	1	0	0	2	1	1	2
7H	GBM1461	1	3	4	4	0	5	1	3	3
	GBM1464	0	0	5	1	1	1	4	2	1
	GBM1516	0	1	2	2	0	2	1	0	4
Total		5	12	32	14	3	31	13	14	36

Table 4. Diversity index (PIC value) at e-SSR loci

Chromosome and marker		Geographical region					
		Austria		Albania		India	
		1922–1932	1982	1941	1994	1937	1976
1H	GBM1002	0.28	0.44	0.41	0.50	0.48	0.00
	GBM1007	0.64	0.78	0.46	0.72	0.57	0.65
	GBM1461	0.74	0.83	0.83	0.72	0.57	0.75
2H	GBM1208	0.00	0.56	0.00	0.12	0.73	0.55
	GBM1218	0.18	0.00	0.66	0.70	0.39	0.46
3H	GBM1059	0.71	0.70	0.86	0.64	0.78	0.75
	GBM1280	0.55	0.65	0.58	0.40	0.59	0.68
4H	GBM1020	0.50	0.50	0.50	0.49	0.49	0.49
	GBM1221	0.54	0.54	0.47	0.21	0.71	0.76
6H	GBM1404	0.00	0.00	0.20	0.20	0.00	0.15
7H	GBM1419	0.00	0.00	0.11	0.29	0.44	0.57
	GBM1464	0.70	0.75	0.10	0.15	0.70	0.35
	GBM1516	0.15	0.54	0.56	0.48	0.78	0.66
Mean		0.38	0.48	0.44	0.43	0.56	0.52

materials collected in the two missions (1941 and 1994), the total number of alleles was slightly (about 15%) lower in the material from the successive collection mission. Overall, it seems that there has been a real loss of genetic diversity in the materials collected in Albania in 1994, compared to 1941. This decrease may be driven by the economic and political isolation of Albania after the Second World War.

Our results are suggestive of an overall stability in the genetic diversity in the barley genebank accessions collected up to 80 years ago in three diverse geographic regions, and the material that entered the genebank about 40–60 years later, originating from more or less the same areas. These results are in accordance with those observed in the study of Backes *et al.* (2003), who compared the diversity of 37 landraces with that of 76 old (released before 1975) and 66 recent cultivars divided into two-rowed spring and six-rowed winter barleys. No significant change was detected. Also Koebner *et al.* (2003) concluded that systematic barley breeding in the UK has not resulted in any reduction of genetic

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Table 5. Chromosomal location and numbers of collection mission-specific and shared alleles for g-SSR loci

Chromosome	and marker	Geographical region								
		Austria			Albania			India		
		No. of specific alleles			No. of specific alleles			No. of specific alleles		
		1922–1932	1982	No. of shared alleles	1941	1994	No. of shared alleles	1937	1976	No. of shared alleles
1H	GBMS0184	1	2	2	2	1	4	1	0	4
	Bmag0211	1	1	3	3	0	3	0	1	8
	Bmag0718	0	2	4	2	1	5	2	0	4
	Bmac0032	2	1	4	8	3	5	8	5	4
2H	Bmag0518	2	0	4	2	1	3	2	4	7
	GBMS0247	1	0	3	4	0	3	2	5	5
	GBMS0160	1	0	2	0	0	3	0	2	4
	HVM0036	2	1	3	1	2	3	0	4	3
3H	Bmag0013	0	2	5	6	0	5	2	4	8
	Bmag0225	3	3	5	4	0	6	1	3	5
	GBMS0189	3	1	6	4	3	4	4	8	3
	Bmag0603	2	2	3	3	3	5	2	2	6
4H	GBMS0133	0	1	3	2	1	3	0	1	4
	EBmac0701	3	1	4	6	0	5	4	1	4
	GBMS0087	2	0	2	1	1	3	3	1	3
	EBmac0788	4	1	3	6	0	3	4	2	4
5H	GBMS0032	2	2	3	4	1	2	1	1	2
	EBmac0684	0	0	4	1	0	4	1	2	2
	CBMS0119	1	2	2	2	1	2	3	4	2
	GMS0001	2	1	2	2	0	3	1	2	2
6H	Bmac0316	3	0	3	3	0	3	4	4	2
	Bmac0040	3	3	5	7	5	6	5	8	4
	EBmac0602	0	2	1	4	1	1	3	3	5
	Bmag0613	3	3	3	2	3	3	5	2	7
7H	GBMS0192	1	0	4	1	0	4	1	1	2
	GBMS0035	0	0	3	2	1	1	2	0	3
	EBmac0755	2	1	4	3	1	6	1	0	5
	Bmag0135	0	2	6	3	1	5	7	2	3
Total		44	34	96	88	30	103	69	72	115

diversity. On the other hand, Russell *et al.* (2000) documented a decrease in the level of diversity (from 0.60 to 0.48) in comparisons between landraces and modern cultivars.

Nevertheless, the present results suggest that genetic diversity within barley landraces has been maintained in the period since genebank activities commenced in the first half of the last century. Notwithstanding this observation, qualitative changes have still occurred. For instance, 14–40% of the alleles at g-SSR loci and 6–29% of alleles at e-SSR loci were unique for the period of collection. This indicates a significant degree of qualitative change in the overall genetic diversity. We suggest this may be the result of an allele flow which occurred during the transition between traditional and modern agricultural systems. Similar results have also been found for European barley cultivars released in the various decades of the 20th century (Malysheva-Otto *et al.*, 2004).

Higher level of polymorphism displayed by g-SSR than e-SSR markers

More alleles (mean 7.7 alleles per marker), as well as a higher PIC value (0.67 per marker), were achieved using the g-SSR markers compared to the levels generated by the e-SSR markers (4.1 alleles per marker, mean PIC value 0.47). A similar result has also been reported elsewhere (Pillen *et al.*, 2000; Russell *et al.*, 2004), and is attributed to the fact that e-SSRs are derived from coding sequence, which is likely to be more highly conserved than non-coding sequence (Varshney *et al.*, 2005). However, since e-SSRs are present within genes, they tend to produce higher-quality, more robust markers, and furthermore, in contrast to g-SSRs, since they are associated with a putative function, they represent a better platform for the assessment of functional diversity (Kota *et al.*, 2001; Eujayl *et al.*, 2002; Thiel *et al.*, 2003).

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Genetic diversity in crop plants

The conclusions we have described here for barley are consistent with those derived from studies of other crops. Investigating wheat accessions from the same collection missions as well as from additional missions in Nepal, Khlestkina *et al.* (2004a) were not able to find any significant difference between the missions either for allele number or for PIC value. Other studies examining the genetic diversity of wheat varieties produced during the last century in various geographical regions or breeding programmes have also revealed negligible evidence for any quantitative change resulting from breeding activity (Gregova *et al.*, 1997; Donini *et al.*, 2000; Manifesto *et al.*, 2001; Christiansen *et al.*, 2002; Khlestkina *et al.*, 2004b; Roussel *et al.*, 2004). As well as analyses in the cereals, similar studies have also been undertaken in common bean (*Phaseolus vulgaris* L.). Thus in a comparison between landraces collected in Slovenia in the 1950s and newly acquired material, no complete allele loss could be demonstrated (Sustar-Vozlic *et al.*, 2004). Once again, over time, a qualitative rather than a quantitative shift in diversity was observed. Overall, it appears that human interventions, such as modern agricultural systems and the breeding of high-yielding varieties, do not necessarily lead to any reduction in the genetic diversity of crop plants. Instead of a quantitative shift, the general pattern is one of qualitative changes.

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