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Comparative Assessment of the Genetic Variation in Wild and Cultivated Barley Based on SSR Markers

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ABSTRACT

Nineteen SSR primer pairs were used to assess the genetic diversity of 13 wild populations of *H. vulgare* ssp. *spontaneum* K. (*Hs*) from Israel and Turkmenistan and 68 cultivated barley varieties (*H. vulgare* ssp. *vulgare* L. (*Hv*)). A total of 254 alleles from 22 SSR loci were revealed. The results were: a) Nei's index of genetic variation for wild barley (He=0.71) was higher than for cultivated barley (He=0.63); b) the level of genetic variation of cultivars from Kazakhstan was higher (He=0.68) than European samples (He=0.52); c) of the total genetic diversity of *Hordeum vulgare* L., 69.83% was within populations, 9.28% between populations within a subspecies, and 20.89% between subspecies; d) the structure of genetic diversity for *Hs* was 36.10% within populations, 50.16% between populations of a region, and 13.74% between regions. The results confirmed high potential of SSR markers for genetic diversity analysis and efficient identification of wild and cultivated barley genotypes.

Keywords: DNA, genetic diversity, *Hordeum vulgare* ssp. *vulgare* L., *Hordeum vulgare* ssp. *spontaneum* K., microsatellite
Abbreviations: EU, European Union; ***Hs***, *Hordeum vulgare* ssp. *spontaneum* K.; ***Hv***, *Hordeum vulgare* ssp. *vulgare* L.; **KZ**, Kazakhstan; **SSR**, simple sequence repeat

INTRODUCTION

Cultivated barley (*H. vulgare* ssp. *vulgare* L. (*Hv*)) is one of the most important grain crops and represents a valuable source for human and animal food and beer production (Poehlman 1985). Due to the importance of this crop, breeders have utilized various approaches to improve the quantitative and qualitative characteristics of newly released cultivars. One of the ways to improve productivity of cultivated barley is to utilize the genetic pool of *H. vulgare* ssp. *spontaneum* K (*Hs*), the direct ancestor of cultivated barley (Nevo 1992). The analysis of barley populations from the Near East allowed the identification of a number of accessions, showing increased resistance to stress and diseases (Nevo *et al*. 1986). This was the result of almost 20 years of research on the genetic variation of populations from the Fertile Crescent (Nevo 1992). However, relevant information is not fully available for populations from northern and eastern parts of the *Hs*' habitat (e.g. Turkmenistan), which prevents a more thorough and complete understanding of the extent and structure of genetic variation in *Hs* and its relationship to ecological and environmental factors. Additionally, the reduction of genetic diversity within cultivated species as a result of modern agricultural practices requires a careful re-evaluation of germplasm collections with the main objective of revealing valuable genetic diversity. An adequate detailed analysis of the genetic variability in germplasm relies on the utilization of reliable genetic markers. During the last 2-3 decades, several types of DNA markers have been developed for genome analysis and genotyping (Dudley 1993). Simple sequence repeats (SSRs), or microsatellites, have been used in both barley genome mapping and genetic diversity studies (Ramsay *et al.* 2000; Karakousis *et al.* 2003; Malysheva-Otto *et al.* 2006; Varshney *et al.* 2006; Stein *et al.* 2007). SSR analysis has several advantages over the other molecular marker systems. These advantages include a high level of polymorphism, codominant type of inheritance, assay simplicity, and marker stability (Agarwal *et al.* 2008). Furthermore, for the species, including barley, for which detailed molecular maps are available, SSRs markers can be easily selected based on their chromosomal location and level of polymorphism (Donini *et al*. 1998).

The purposes of this research were (i) to assess the genetic variation of barley cultivars from Kazakhstan, (ii) to assess and compare the extent and structure of the genetic diversity present in wild and cultivated barley, and (iii) to explore SSR markers for DNA genotyping of cultivated barley.

MATERIALS AND METHODS

Ninety six *Hs* plants, representing 4 populations collected in Israel and 9 from Turkmenistan, collected in 1994 (Turuspekov *et al*. 1996), and 68 barley cultivars representing the major barley growing regions in Europe (EU, 34 cultivars) and Kazakhstan (KZ, 34 cultivars, **Table 1**) were selected for SSR analysis. DNA samples were extracted according to Milligan (1992). Barley SSR markers (**Table 2**) were developed at SCRI, Dundee, UK (Macaulay *et al*. 1998) and Virginia Tech, VA, USA (Liu *et al*. 1994). The selected SSR markers covered all seven barley chromosomes (**Table 2**) and previously successfully employed for the description of the genetic variation in Japanese cultivars (Turuspekov *et al.* 2001). Therefore, the same set of SSR markers was used for genetic diversity assessment in EU and KZ lines. Also it made it a lot easier to compare obtained data with those reported earlier for Japanese barley cultivars. PCR reactions were as follows: 30 cycles of denaturing at 94°C for 1 min, annealing at 55 or 60°C for 1 min, extension at 72°C for 1 min followed by a single extension at 72°C for 7 min. PCR products were separated using 6% polyacrylamide gel electrophoresis and revealed by silver staining as described by Wade-Evans (1996). Statistical analysis was performed using POPGENE software (version 1.31), utilizing Nei's genetic diversity index (Nei 1972) and Shannon's information index (Shannon and Weaver 1949). Partitioning of the total genetic diversity was calculated according to Lewontin (1972).

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Table 1 List of barley varieties from Europe and Kazakhstan.

Europe	Year of release	Ear type	winter / spring*	Kazakhstan	Year of release	Ear type	winter / spring*
Alexis	1985	2	s	Arna	1993	2	s
Aramir	1974	2	s	Baisheshek	1978	2	s
Arda	1985	2	w	Bereke54	1994	2	w
Arma	1975	6	w	Chernigovsky5	1962	2	s
Cornell	1976	6	s	Donetsky8	1979	2	s
Corona	1980	6	w	Fermer	-	2	w
Etrusco	1981	6	w	Granal	1988	2	s
Express	1985	6	w	Karabalyksky43	1991	2	s
Franka	1980	6	w	Karabalyksky150	1991	2	s
Georgie	1977	2	s	Karabalyksky179	1992	2	s
Gerbel	1977	6	w	Karagandinsky4	1988	2	s
Gimpel	1979	2	s	Kharkovsky99	1991	2	s
Grit	1979	2	s	Kompleksny	1997	2	s
Havila	1979	2	s	Medicum85	1986	2	s
Igri	1977	2	w	Medicum8955	1941	2	s
Jaidor	1981	6	w	Mereke150	1996	2	s
Kenia	1931	2	s	Nutans187	1845	2	s
Marinka	1985	2	w	Odessky100	1985	2	s
Massa	-	6	w	Omsky87	1981	2	s
Opale	1979	6	w	Pastbishny	1991	2	s
Pirate	1978	6	w	Roman	1984	2	w
Porthos	1975	2	s	Rosava	1986	2	w
Prisma	1986	2	s	Saule	1987	2	s
Protidor	1981	2	a	Sever1	1996	2	s
Regatta	1987	2	s	Solontsovy1	1985	2	s
Rika	1951	2	s	Taina	1989	2	w
Robur	1973	6	w	Tobol	1996	2	s
Tipper	1981	2	w	Toguzak	1990	2	s
Trebbia	1990	6	w	Tselinny5	1988	2	s
Triumph	1973	2	s	Tselinny30	1971	2	s
Tyne	1988	2	s	Tselinny91	1991	2	s
Vogelsanger Gold	1965	6	w	Tsiklon	1985	2	w
Wisa	1951	2	w	Ubagan	1996	2	s
Zephyr	1975	2	s	Zhyldyz	1989	2	s

*w: winter, s: spring

Table 2 Genetic diversity of groups of wild barley based on SSR analysis.

Populations		**Isr**	**Tr**	***Hs***	**Isr**	**Tr**	***Hs***	**Isr**	**Tr**	***Hs***
No of plants		**21**	**75**	**96**	**21**	**75**	**96**	**21**	**75**	**96**
Marker	**Chromosome**	**Number of alleles**			**Nei's genetic diversity index**			**Shannon index**		
SCRI										
Bmac 0018	6H	4	5	5	0.67	0.72	0.81	1.19	1.34	1.45
Bmac0030	4H	2	4	4	0.25	0.70	0.77	0.41	1.29	1.19
Bmac0032	1H	11	11	17	0.88	0.83	0.89	2.27	2.01	2.28
Bmac0040	6H	9	9	14	0.82	0.83	0.92	1.92	1.94	2.31
Bmac0067	3H	10	12	16	0.83	0.85	0.89	2.02	2.14	2.47
Bmac0209	3H	6	6	8	0.77	0.68	0.79	1.59	1.38	1.52
Bmac0213-1	1H	1	2	2	0.00	0.08	0.49	0.00	0.17	0.56
Bmac0213-2	1H	6	7	8	0.79	0.66	0.87	1.66	1.38	1.68
Bmac0218-1	2H	9	12	16	0.86	0.86	0.87	2.05	2.18	2.40
Bmac0218-2	2H	8	9	14	0.36	0.76	0.89	1.82	1.73	2.12
Bmac0218-3	6H	2	2	2	0.36	0.48	0.48	0.55	0.68	0.69
Bmac0316	6H	8	8	11	0.82	0.79	0.87	1.87	1.73	1.96
EBmac0541	3H	6	7	11	0.79	0.53	0.80	1.66	1.13	1.62
Bmag0005	5H	5	6	8	0.77	0.71	0.69	1.53	1.42	1.76
Bmag0006	3H	10	10	13	0.87	0.79	0.87	2.16	1.85	2.15
Bmag0140	2H	8	3	9	0.82	0.49	0.76	1.87	0.73	1.41
Bmag0206	7H	6	9	11	0.80	0.71	0.87	1.69	1.49	1.81
Bmag0321	7H	6	5	9	0.79	0.65	0.75	1.66	1.22	1.67
VT										
HVKASI	2H	5	4	7	0.77	0.63	0.78	1.50	1.09	1.64
HVCMA	7H	2	3	3	0.41	0.51	0.66	0.60	0.79	0.78
HVLEU	5H	2	1	2	0.09	0.00	0.28	0.19	0.00	0.06
HVCABG	7H	7	6	10	0.81	0.73	0.88	1.77	1.54	1.96
Mean		6	6.4	9.1	0.67	0.64	0.77	1.45	1.33	1.61
St. Dev.		3	3.3	4.7	0.26	0.22	0.16	0.66	0.58	0.63

SCRI: Scottish Crop Research Institute, VT: Virginia Polytechnic Institute and State University,
Isr: Israel, Tr: Turkmenistan, *Hs*: *Hordeum vulgare* ssp *spontaneum*

RESULTS

Nei's genetic variation indices for *Hs* populations and *Hv* cultivars based on frequencies of SSR alleles are presented in **Tables 2** and **3**. In general, Nei's index of genetic variation for wild barley was higher (He=0.71) than for cultivated barley (He=0.63). According to their level of polymorphism the SSR loci could be divided into three groups: (i) low polymorphic (0 – 2 alleles, with a Shannon Index between 0 and 1: Bmac0213(1), Bmac0218(3), and HVLEU); moderately polymorphic (3-12 alleles, with a Shannon Index between 1 and 2: Bmag0140, HVKASI, Bmac0209, EBmac0541, Bmac0030, Bmag0005, Bmac0018, HVCMA and Bmac0321); and highly polymorphic (13-21 alleles, with a Shannon Index above 2: Bmac0213(2), Bmac0032, Bmac0067, Bmag0006, HVCAB, Bmac0316, Bmac0218(1), Bmac0218(2), Bmac0040 and Bmag0206). The SSRs characterized by a Shannon Index > 1 seem suitable for DNA fingerprinting of cultivated barley. For instance, **Fig. 1** shows that only four SSR markers were suf-

Table 3 Genetic diversity of groups of cultivated barley based on SSR analysis.

	KZ	EU	*Hv*	KZ	EU	*Hv*	KZ	EU	*Hv*
No of plants	**34**	**34**	**68**	**34**	**34**	**68**	**34**	**34**	**68**
Marker	**Number of alleles**			**Nei's genetic diversity index**			**Shannon index**		
SCRI									
Bmac 0018	3	3	3	0.48	0.60	0.55	0.75	0.97	0.89
Bmac0030	5	4	5	0.68	0.52	0.62	1.32	0.96	1.19
Bmac0032	8	6	10	0.74	0.74	0.77	1.61	1.53	1.73
Bmac0040	13	12	15	0.88	0.80	0.88	2.33	1.99	2.36
Bmac0067	7	4	7	0.71	0.66	0.76	1.53	1.22	1.55
Bmac0209	8	4	9	0.81	0.55	0.72	1.78	0.95	1.56
Bmac0213-1	2	2	2	0.49	0.33	0.44	0.69	0.51	0.63
Bmac0213-2	12	11	14	0.86	0.78	0.86	2.15	1.86	2.26
Bmac0218-1	6	4	7	0.68	0.22	0.51	1.37	0.48	1.11
Bmac0218-2	9	5	11	0.81	0.65	0.79	1.87	1.25	1.89
Bmac0218-3	2	2	2	0.44	0.29	0.38	0.63	0.47	0.56
Bmac0316	12	4	13	0.69	0.55	0.65	1.71	0.91	1.47
EBmac0541	4	3	4	0.62	0.21	0.47	1.10	0.43	0.88
Bmag0005	5	3	5	0.53	0.21	0.39	1.09	0.43	0.84
Bmag0006	7	4	7	0.80	0.60	0.75	1.75	1.03	1.57
Bmag0140	4	2	4	0.66	0.25	0.50	1.21	0.42	0.93
Bmag0206	8	4	8	0.82	0.69	0.78	1.86	1.38	1.76
Bmag0321	8	6	9	0.83	0.57	0.74	1.89	1.15	1.67
VT									
HVKASI	4	4	5	0.60	0.55	0.58	1.04	0.92	1.02
HVCMA	3	2	3	0.49	0.36	0.43	0.79	0.55	0.69
HVLEU	2	2	2	0.47	0.48	0.48	0.67	0.67	0.67
HVCAB	9	9	10	0.86	0.82	0.86	2.04	1.90	2.06
Mean	6	4.6	7.1	0.68	0.52	0.63	1.42	1.00	1.33
St. Dev.	3	2.8	4	0.14	0.19	0.16	0.52	0.50	0.55

SCRI: Scottish Crop Research Institute, VT: Virginia Polytechnic Institute and State University, KZ: Kazakhstan, EU: Europe, *Hv: Hordeum vulgare* ssp *vulgare*

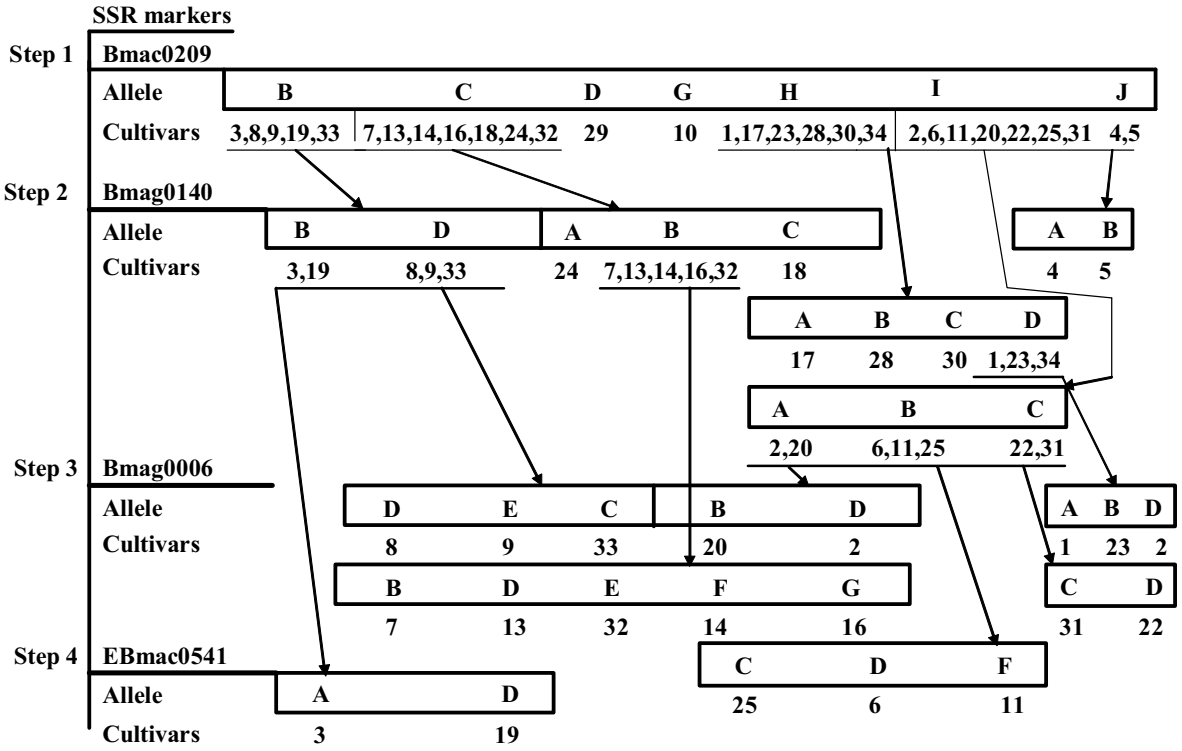

Fig. 1 Identification steps for 34 barley varieties from Kazakhstan based on four SSR markers. Letters in blocks represent SSR alleles; numbers under blocks represent barley cultivars listed in **Table 1**.

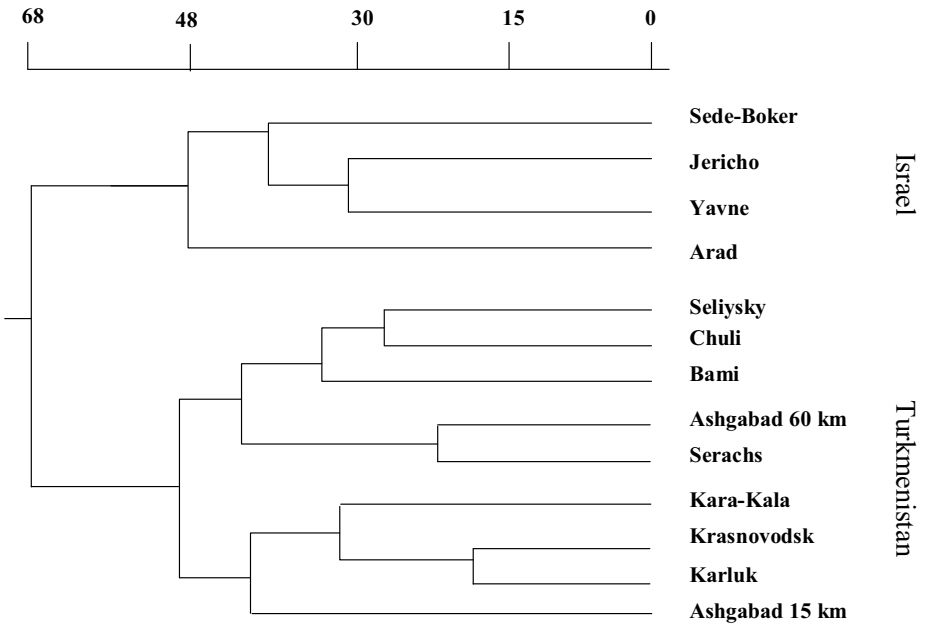

Fig. 2 Cluster analysis of Nei's genetic distance values based on SSRs allele frequencies in 13 wild barley populations. Scale of length computed based on Nei's genetic distance using UPGMA. Dendrogram constructed using Popgene software (version 1.32).

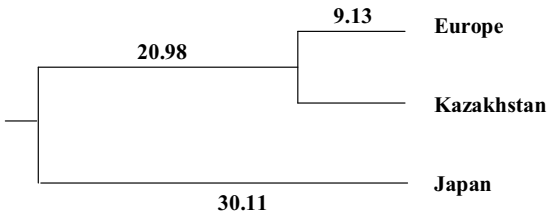

Fig. 3 The dendrogram represents genetic similarities of barley cultivars grown in Europe, Kazakhstan and Japan and constructed based on analysis of SSR markers (Popgene software, version 1.32). Scale of length computed based on Nei's genetic distance using UPGMA.

Table 4 Genetic similarity among barley cultivars of Europe, Kazakhstan and Japan based on Nei's genetic distance (below diagonal) and genetic diversity (above diagonal). Computed using SSR allele frequencies and Popgene software (version 1.32)

Regions	Europe	Kazakhstan	Japan
Europe	****	0.83	0.54
Kazakhstan	0.18	****	0.55
Japan	0.61	0.59	****

ficient enough to reliably identify barley lines from a sample of 34 KZ cultivars. Several SSR alleles, like allele "B" from locus Bmac0213(1), allele "F" from Bmag0140, alleles "C" and "D" from Bmac0067, allele "D" from EBmac0541, allele "D" from Bmag0206, etc., were present at high frequency in the wild populations of *Hs* collected in Turkmenistan and were not found in those collected in Israel, and also detected with low frequency or were completely absent among cultivated barley lines.

Fourteen cultivars released before 1975 were grouped separately from those released after 1975. However, Nei's index was similar for both groups (0.61 and 0.63, respectively).

The genetic distance ranged from 0.18 between the groups of EU and KZ cultivars to 1.20 between EU cultivars and Turkmenistan wild populations, whereas for *Hs* it ranged from 0.32 between the stations near Krasnovodsk and Karluk in Turkmenistan to 2.02 between the stations near Ashgabad (15 km) in Turkmenistan and Jericho in Israel. The phylogenetic tree based on microsatellite data for *Hs* accessions (**Fig. 2**) clearly separated the populations sampled in Israel from those originated from Turkmenistan. Within regions, the genetic distance was not related to their geographic distance. For example, populations that are very distant geographically (e.g. Krasnovodsk and Karluk) appear to be very close genetically, and *vice versa* (e.g. Ashgabad 15 km and Ashgabad 60 km, **Fig. 2**).

Previously, by using the same set of SSR markers we reported about genetic diversity among barley cultivars from Japan (Turuspekov *et al.* 2001). The comparison of the results for the genetic diversity between three regions demonstrated a strong link between KZ and EU cultivars (0.83), while Japanese cultivars showed significantly less similarity to both KZ (0.55) and EU cultivars (0.54, **Table 4, Fig. 3**). The level of genetic variation (Nei's index) within *Hv* cultivars was highest in Kazakhstan (0.68) followed by EU (0.52, **Table 3**) and Japan (0.48).

The total variation for barley was partitioned as follows: 20.9% between subspecies, 9.3% among populations within a subspecies and 69.8 within populations. Only 9.7% of the total variation within *Hv* was explained by differences between the groups of EU and KZ's barley cultivars, whereas 90.3% of variation was among cultivars within a region. The total genetic diversity for *Hs* was divided as follows: 36.1% within populations, 50.2% among populations within a region, and 13.7% between regions. In Israeli populations partitioning was 45% within and 55% between populations, in Turkmenistan populations it was 38 and 62%, respectively.

DISCUSSION

SSR variation and DNA fingerprinting of barley cultivars

Obtained results confirmed the high level of SSR variability in cultivated barley as was also shown in earlier reports (Russel *et al.* 1997; Donini *et al.* 1998; Struss and Plieske 1998). On average, we observed 11.6 alleles per SSR locus, while Struss *et al.* reported 8.06 and 4.82 alleles per locus, respectively. The mean value of genetic diversity in our study (0.77) was similar to that reported by Struss and Plieske in 1998 (0.73).

In KZ, over the last decades little effort has been made for the reliable DNA fingerprinting of barley cultivars. Recent achievements in developing PCR-based markers have significantly reduced the cost of DNA analysis and simpli-

fied the approach for fast and effective sample identification. In this study only four SSR markers (**Fig. 1**) were sufficient enough to identify each barley accession from a sample of 34 cultivars from KZ. The majority of examined lines were identified just by genotyping with three SSR markers. These results can be employed for the reliable genotyping of all cultivated barley accessions in KZ.

Earlier we reported the use of the same SSR markers to discriminate barley cultivars grown in different regions of Japan (Turuspekov *et al*. 2001). Therefore, the results obtained in this work can be used for the assessment of genetic diversity of cultivated barley collected from three different regions; EU, KZ (Central Asia), and Japan (Far East). The genetic comparison of the results among barley cultivars grown in different regions indicates stronger genetic similarity between EU and KZ cultivars in comparison with cultivars from Japan (**Table 4**). Since KZfor many decades has been a part of the former USSR and has strong cultural and scientific links with European countries, the result was not an un-expected outcome. Although it is speculated that the Japanese barley cultivars were bred from European germplasm (Azhaguvel and Komatsuda 2007) it is obvious that they had a greater influence from other continents when compared to cultivars from KZ.

Extent and structure of genetic diversity

Although the genetic variation of wild barley has been found to be extensive (Nevo 1992), the possibility of genetic erosion within cultivated species, as a result of both domestication bottlenecks and modern breeding practices, is a major concern. However, results obtained in this study demonstrated that genetic diversity of commercial barley lines from KZ is in the same range as one revealed among *Hs* accessions. Nei's index of genetic variation for barley cultivars from KZ was 0.68 while in *Hs* from Israel and Turkmenistan was 0.76 and 0.64, respectively. The wide range of environmental conditions in which barley cultivation is actually taken place in EU and KZ, has probably favored a high level of genetic variation that helps to provide an adequate environmental adaptability. In addition, the level of genetic diversity among older cultivars released before 1975 was similar to that observed among more recently released cultivars.

The structure of *H. vulgare* L., in this particular research, indicates that only 21% of the total variation was accounted for by differences between the two subspecies. The structure of the genetic diversity present in *Hs* evidenced in this study appeared to be different from the results obtained with isozymes analysis (Turuspekov *et al*. 1996; Volis *et al*. 2000). While isozyme analysis showed that most of the genetic variation was concentrated within populations, SSRs indicated that more than 50% of the total variation derived from differences between populations within a region. This result, if confirmed by further studies, may lead to the alternation of sampling strategies for wild barley plants with a focus on the collection of plants in as many different sites within a region as possible (Marshall and Brown 1975; Owuor *et al.* 1997).

Use of SSR markers in genetics and breeding of barley

The development and use of SSR markers is one of the exciting chapters in further discovering of barley genome. Recent data mining is suggesting that potentially more than 2800 SSRs can be used for further genomics studies in barley community (Varshney *et al.* 2006). The available results based on using SSR markers can be separated in three groups. The first group of results is dedicated to the description of the genetic diversity both in cultivated and wild barley (Maroof *et al.* 1994; Struss and Plieske 1998; Ivandic *et al.* 2002). Our work is an additional input in this category and confirms that SSRs can provide a reliable discriminatory resource for the assessment of genetic diversity in barley. The second group of results is falling to the construction of detailed genetic maps of barley (Ramsay *et al.* 2000), including the integration of barley mapping efforts from various groups around the world (Li *et al.* 2003; Varshney *et al.* 2007) and comparative mapping of barley with related genomes of cereal species (Holton *et al.* 2002; Varshney *et al.* 2005). The third group of results is an attempt to improve marker-directed plant breeding via reliable DNA tagging of specific resistant genes to biotic (Werner *et al.* 2003; Bai and Shaner 2004) and abiotic stresses (Forster *et al.* 2000; Raman *et al.* 2002), and DNA genotyping of barley accessions (Karakousis *et al.* 2003). Overall, it seems that the SSR technology is increasingly emerging as a one of the major marker tools in research communities all over the world.

CONCLUSIONS

SSR markers are often referred as one of the most informative and efficient types of DNA markers and, therefore, were used in this study. The results of this work show a high level of genetic variation in barley cultivars from KZ, which is comparable with the genetic variation in populations of wild barley. The results suggested that barley accessions from KZ have closer genetic similarity to the EU gene pool when compared with Japanese cultivars. Four SSR markers were enough to successfully discriminate all 34 cultivars from KZ used in this work and, therefore, they can be recommended to be included to the core set of DNA markers for the purpose of genotyping barley germplasm in KZ. Analysis of the partitioning of genetic variation in wild populations of Israel and Turkmenistan suggests that about 50% of variation is among populations within a region. This result can be helpful for the further optimization of the sampling strategy for wild barley plants. Overall, the obtained results confirmed that SSRs can provide a reliable discriminatory resource for the assessment of genetic diversity in barley.

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REFERENCES

Azhaguvel P, Komatsuda T (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis locus indicates a diphyletic origin of barley. *Annals of Botany* **100**, 1009-1015

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports* 27, 617-631

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. *Annual Review of Phytopathology* **42**, 135-161

Donini P, Stephenson P, Bryan GJ, Koebner R (1998) The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. *Genetic Resources and Crop Evolution* **45**, 415-421

Dudley JW (1993) Molecular markers in plant improvement: manipulation of genes affecting of quantitative traits. *Crop Science* **33**, 660-668

Forster BP, Ellis RP, Thomas WTB, Newton AC, Tuberosa R, This D, El-Enein RA, Bahri MH, Ben Salem M (2000) The development and application of molecular markers for abiotic stress tolerance in barley. *Journal of Experimental Botany* **51**, 19-27

Holton TA, Christopher JT, McClure L, Harker N, Henry RJ (2002) Identification and mapping of polymorphic SSR markers from expressed sequences of barley and wheat. *Molecular Breeding* **9**, 63-71

Ivandic V, Hackett C, Nevo E, Keith R, Thomas W, Forster B (2002) Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. *Plant Molecular Biology* **48**, 511-527

Karakousis A, Barr AR, Chalmers KJ, Ablett GA, Holton TA, Henry RJ, Lim P, Langridge P (2003) Potential of SSR markers for plant breeding and variety identification in Australian barley germplasm. *Australian Journal of Agricultural Research* **54**, 1197-1210

Lewontin RC (1972) The apportionment of human diversity. *Evolutionary Biology* **6**, 381-398

Li JZ, Sjakste TG, Röder MS, Ganal MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. *Theoretical and Applied Genetics* **107**, 1021-1027

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theoretical and Applied Genetics* **93**, 869-876

Macaulay M, Ramsay L, Russell J, Mclean K, Fuller J, Powell W, Waugh R (1998) Development and application of SSR-based markers in barley. *Plant and Animal Genome VI Conference,* San Diego, CA, USA, p 194 (Abstract)

Malysheva-Otto L, Ganal M, Roder M (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). *BMC Genetics* **7 (6)**, 1-14

Marshall DR and Brown AHD (1975) Optimum sampling strategies in genetic conservation. In: Frankel O, Hawks J (Eds) *Crop Genetic Resources for Today and Tomorrow*, Cambridge University Press, pp 53-70

Milligan B (1992) Plant DNA isolation. In: Hoelzel R (Ed) *Molecular Genetics Analysis of Populations*, Oxford University Press, pp 59-88

Nei M (1972) Genetic distance between populations. *American Naturalist* **106**, 283-292

Nevo E (1992) Origin, evolution, population genetics, and resources for breeding of wild barley, *H. spontaneum*, In Fertile Crescent. In: Shewry PR (Ed) *Barley: Genetics, Biochemistry, Molecular Biology, and Biotechnology*, CAB International, Wallingford, pp 19-43

Owuor E, Kimani PM, Mendlinger S, Chweya J, Whittaker L (1996) Comparative allozymic multilocus analysis of genetic diversity in wild barley from Turkmenistan (former USSR) and Israel: implications on sampling strategies. *Barley Genetic Newsletters* **25**, 10-13

Poehlman JM (1985) Adaptation and distribution. In: Rasmusson DC (Ed) *Barley*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, USA, pp 2-12

Raman H, Moroni JS, Sato K, Read BJ, Scott BJ (2002) Identification of AFLP and microsatellite markers linked with an aluminium tolerance gene in barley (*Hordeum vulgare* L.) *Theoretical and Applied Genetics* **105**, 458–464

Ramsay L, Macaulay M, Ivanissevich SD, Maclean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal MW, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. *Genetics* **156**, 1997-2005

Russel J, Fuller J, Young G, Thomas B, Taramino G, Macaulay M, Waugh R, Powell W (1997) Discriminating between barley genotypes using microsatellites markers. *Genome* **40**, 442-450

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proceedings of the National Academy of Sciences USA* **91**, 5466-5470

Shannon CE, Weaver W (1949) *The Mathematical Theory of Communication*, University of Illinois Press, Urbana, 117 pp

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. *Theoretical and Applied Genetics* **114**, 823-839

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. *Theoretical and Applied Genetics* **97**, 308-315

Turuspekov Y, Abugalieva S, Mendlinger S, Volis S (1996) Polymorphism in wild barley (*H. spontaneum* K.) populations from Turkmenistan. *Russian Journal of Genetics* **32 (6)**, 767-773

Turuspekov Y, Nakamura K, Tuberosa R (2001) Genetic diversity of Japanese barley varieties based on SSR analysis. *Breeding Science* **51**, 215-218

Varshney RK, Sigmund R, Börner A, Korzun V, Stein N, Sorrells ME, Langridge P, Graner A (2005) Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. *Plant Science* **168**, 195-202

Varshney RK, Grosse I, Haehnel U, Siefken R, Prasad M, Stein N, Langridge P, Altschmied L, Graner A (2006) Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome. *Theoretical and Applied Genetics* **113**, 239-250

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. *Theoretical and Applied Genetics* **114**, 1091-1103

Volis S, Mendlinger S, Turuspekov Y, Esnazarov U (2002) Phenotypic and allozyme variation in Mediterranean and desert populations of wild barley, *Hordeum spontaneum* Koch. *Evolution* **56**, 1403-1415

Wade-Evans A (1996) Silver Sequencing™: Nonradioactive cycle sequencing of dsDNA. In: Rapley R (Ed) *PCR Sequencing Protocols*, Humana Press, New Jersey, pp 91-101

Werner K, Friedt W, Laubach E, Waugh R, Ordon F (2003) Dissection of resistance to soil-borne yellow-mosaic-inducing viruses of barley (BaMMV, BaYMV, BaYMV-2) in a complex breeders' cross by means of SSRs and simultaneous mapping of BaYMV/BaYMV-2 resistance of var. "Chikurin Ibaraki 1". *Theoretical and Applied Genetics* **106**, 1425-1432