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Determining the levels of genetic variation using SSR markers in three Turkish barley materials known as Tokak

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Abstract: Genetic variations are the raw materials of plant breeding and have been increasingly narrowed due to various reasons. Landraces are good sources of genetic variation, but need to be characterized first. This study was conducted to determine the levels of genetic variation in 3 barley materials originated in Turkey, all known as Tokak. Of the 3 materials used, PI 470281 is a landrace and CIHO 10093 is a pure line. The third is Tokak 157/37, a major cultivar of Turkey considered to be a landrace. In order to detect the levels of polymorphism within and among these materials 30 SSR (microsatellite) markers were used. Some morphological markers were also studied. Ten CIHO 10093 plants did not show any polymorphism with 30 SSR markers. Only 5 SSR markers yielded polymorphism within the 60 lines of cv. Tokak 157/37 studied. There were only 2 different genotypes. The common genotype was observed in 58 lines and the rare one in 2 lines. This finding shows that Tokak 157/37 has lost its variation and is no longer a landrace. Twenty-three of the 30 SSR markers were polymorphic in PI 470281. The 30 SSR markers produced 70 alleles in 52 of the PI 470281 plants. Among the 52 plants studied, 46 different genotypes were detected, indicating a very high level of polymorphism. No polymorphism was observed for the morphological markers. Moreover, variation in plant characteristics was not very high. These facts indicate that farmers who have been using this landrace for a long time might have performed mass selections based on phenotype within the cultivar. A dendrogram was made using 52 PI 470281 lines (a common type of Tokak 157/37), 2 rare lines of Tokak 157/37, and CIHO 10093. Genetic variation observed at the DNA level, but not at the morphological level, might indicate that, despite the uniform appearance, PI 470281 might have novel alleles of the loci that affect the major agronomic traits.

Key words: Barley, genetic variation, landrace, microsatellite markers

Tokak adlı üç Türk arpa genetik materyalinde SSR belirleyicileri kullanılarak genetik varyasyon düzeylerinin belirlenmesi

Özet: Genetik varyasyonlar bitki ıslahının ham maddeleridir ve çeşitli nedenlerle gittikçe daralmaktadırlar. Yerel çeşitler iyi varyasyon kaynaklarıdırlar, ancak öncelikle karakterize edilmeleri gerekmektedir. Bu çalışma, tümü Tokak adıyla bilinen biri safhat ikisi yerel çeşit üç arpa materyalinin akrabalıklarının ve bu materyaller içerisinde mevcut olan genetik varyasyon düzeylerinin belirlenmesi için yürütülmüştür. Bu materyallerden PI 470281 bir yerel çeşittir. CIHO 10093 Tokak isimli bir saf hattır. Tokak 157/37 ise Türkiye'nin önemli bir yerel arpa çeşididir. Bu materyallerdeki genetik çeşitliliği belirlemek için polimorfizm düzeyi yüksek, kodominant nitelikte 30 SSR (microsatellite) belirleyicisi kullanılmıştır. Aynı zamanda bazı morfolojik belirleyiciler de çalışılmıştır. İncelenen 10 CIHO 10093 bitkisi hiçbir

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polimorfizm göstermemiştir. Çalışılan 30 SSR belirleyicisinden sadece beş tanesi Tokak 157/37 içinde polimorfizm belirlemiş ve sadece iki tip genotip bulunduğunu ortaya koymuştur. Bunlardan yaygın olanı 58 hatta, nadir olanı ise iki hatta gözlenmiştir. Bu sonuç Tokak 157/37'nin varyasyonunu büyük ölçüde kaybettiğinin ve artık bir yerel çeşit olmadığını göstermektedir. Kullanılan 30 SSR belirleyicisinden 23 tanesi PI 470281 materyalinde polimorfizm göstermiştir. Bu 30 belirleyici PI 470281'de toplam 70 allel meydana getirmiştir. Çalışılan 52 PI 470281 hattı arasında 46 farklı genotip belirlenmiştir ki bu genetik çeşitlilik seviyesinin çok yüksek olduğu anlamına gelmektedir. PI 470281 içinde morfolojik belirleyiciler bakımından polimorfizm gözlenmemiştir. Aynı zamanda bitkisel özellikler arasındaki varyasyon da yüksek değildir. Bu durum yerel çeşidi kullanan çiftçilerin geçmişte fenotipe dayalı toplu seleksiyonlar yapmış olabileceğini göstermektedir. PI 470281 materyaline ait 52 hat, Tokak 157/37 çeşidinin yaygın genotipi, iki nadir genotipi ve CIHO 10093 ile dendogram oluşturulmuştur. Bu çalışma sonunda DNA düzeyinde gözlenen fakat morfolojik olarak gözlenmeyen varyasyonlar, üniform görünse de PI 470281 materyalinin önemli tarımsal karakterleri etkileyen genlerin yeni allellerini taşıyabileceğini ortaya koyabilir.

Anahtar sözcükler: Arpa, genetik çeşitlilik, mikrosatellit belirleyicileri, yerel çeşitler

Introduction

Yield and quality traits of crop varieties are improved through plant breeding. Genetic variations are the raw materials of plant breeding; however, certain genetic resources have extensively been used in plant breeding programs and thus the genetic basis of some major crops has been increasingly narrowed. Wild species and landraces offer great advantages for widening the genetic basis in plant breeding programs. Landraces are better for the development of quality traits in this respect because unwanted genes that come along with the gene of interest could be less deleterious when they come from landraces than from wild species.

Landraces are populations that are morphologically similar, but genetically different (Harlan 1975). About 50%-60% of the genetic variation of landraces maintained in gene banks is within the landraces (Parzies et al. 2000). Determination of the level of variation within the landraces is very important for their preservation. In order for landraces to be employed in plant breeding programs their levels of genetic variation should first be determined. Investigations on the landraces of Turkey, one of the centers of the origin of barley, could be very useful for identifying new alleles for yield- and quality-related traits in barley.

DNA markers have been used extensively for plant genetic diversity and identification studies. SSR markers are highly informative because their polymorphism rates are high. They have the advantage of being a PCR marker, i.e. they are fast and relatively cheap to analyze. In addition, SSR markers are distributed all over the genomes (Varshney et al. 2005). All these factors make them the marker of choice for genetic research.

SSR markers have been used to detect the genetic diversity of various barley materials, including wild species (Struss and Plieske 1998; Matus and Hayes 2002), landraces (Struss and Plieske 1998; Hamza et al. 2004; Pandey et al. 2006), and commercial cultivars (Struss and Plieske 1998; Pillen et al. 2000; Maestri et al. 2002; Matus and Hayes 2002; Kolodinska Brantestam et al. 2007). Data from these investigations indicate that SSR markers are good tools for determining genetic variability in barley.

Polymorphic information content (PIC) is a measure of a marker's informativeness. Different PIC values were obtained from marker studies using different genetic materials in barley. Hamza et al. (2004) reported PIC values ranging from 0.068 to 0.78 (mean: 0.45). Matus and Hayes (2002) reported PIC values between 0.08 and 0.94; the mean was 0.79 for *H. spontaneum* and 0.75 for *H. vulgare*. Varshney et al. (2006) studied 185 SSR markers of EST-origin in 5 barley cultivars and 2 germplasms, and noted PIC values between 0.24 and 0.78 (mean: 0.48). Pandey et al. (2006) observed PIC values that varied from 0 to 0.88 (mean: 0.50) for 44 SSR markers investigated in 107 Himalayan hulless barley landraces. Finally, Pillen et al. (2000) screened 30 barley materials using 22 SSR markers and reported PIC values ranging from 0.14 to 0.78 (mean: 0.38).

Tokak is the name used for different Turkish barley materials, one of which is cv. Tokak 157/37, which is considered a landrace (Akar et al. 1999). The US National Plant Germplasm System (NPGS) has a pure line (CIHO 10093) named cv. Tokak (obtained from Turkey) and a landrace (PI 470281) also named Tokak collected from the Sarıkamış region, Kars province, Turkey. The aim of the present study was to determine the level of genetic variation in these 3 materials (Tokak 157/37, CIHO 10093, and PI 470281) and their genetic relationships.

Materials and methods

Genetic materials

Three different genetic materials were used in this investigation. The first was CIHO 10093, a pure line called Tokak maintained at the US NPGS. Ten CIHO 10093 plants were studied. The second material was cv. Tokak 157/37, a cultivar grown on a considerable amount of acreage in Turkey (Akar et al. 1999). As Tokak 157/37 material, 60 plants from a seed lot produced for distribution to farmers were studied. The third material was PI 470281, a landrace also named Tokak, collected from the Sarıkamış region of Turkey and maintained at the US NPGS. Fifty-two PI 470281 plants were studied.

DNA analyses

DNA analyses of lines obtained from single seeds of each of the 3 materials were conducted at the Molecular Biotechnology Laboratory of Gaziosmanpaşa University, Agricultural Faculty, Crop Science Department. DNA was isolated according to Doyle and Doyle (1990). About 200 mg of fresh leaf tissue from 2-3 leaf stage plants were ground in liquid nitrogen in an Eppendorf tube using 1-mL pipette tips. DNA quality and quantity were determined using 1% agarose gel and a spectrophotometer. DNA concentration was adjusted to 50 ng μL^{-1}. Genomic regions flanking SSR sequences were amplified using specific primers via polymerase chain reaction (PCR) (Röder et al. 1998). The PCR reaction volume was 40 µL, consisting of 250 nM each of the 2 primers, 0.2 mM each of the nucleotides, 1.5 mM $MgCl_2$, 0.5 units of *Taq*-DNA polymerase (Promega), and 50-100 ng of genomic DNA as a template. A typical PCR procedure was as follows: 5 min at 94 °C, then 32 cycles of 1 min at 94 °C, 1 min at 50-60 °C (depending on the primer), 1 min at 72 °C, and 5 min at 72 °C.

Low copy and high quality SSR markers with relatively high polymorphic information content were selected based on data provided by the GrainGenes database (GrainGenes 2009) (Table 1). All SSR markers used were specific to barley. Primer sequences were obtained from the GrainGenes database; however, primer sequences for GBMS and GBM markers were kindly provided by Dr. Marion S. Röder and Dr. Andreas Graner, respectively.

PCR products were run on 3% MetaPhor™ agarose gel with 1% TBE buffer. DNA was visualized via ethidium bromide added to the gels, using a gel image system (Vilber Lourmat CN-08). Bands were analyzed using BioCapt v.11.02 software. Relationships between the lines were calculated according to Nei (1978). Dendrograms were prepared using the UPGMA algorithm in POPGENE v.1.31 (Yeh et al. 1997). SSR marker polymorphism rates were determined using PIC values, which were calculated according to the following formula:

$$PIC = 1 - \sum P^2 i,$$

where P*i* is the frequency of i^{th} allele (Anderson et al. 1993).

Morphological observations

The lines were subjected to morphological observations during the 2005-2006 and 2006-2007 growing periods under field conditions in Tokat. Morphological traits such as rachilla length, rachilla pubescence, sterile floret size, awn roughness, and anthocyanin pigment in leaves were observed.

Results

CIHO 10093 material

Ten CIHO 10093 plants were analyzed using 30 SSR markers. All the plants had the same profile (data not given) for the SSR markers studied, and the plants did not show any polymorphism for morphological markers. They had long and very pubescent rachillae, well-developed sterile florets, rough awns, and leaves with anthocyanin. None of the SSR markers analyzed were heterozygous.

Tokak 157/37 material

In total, 60 cv. Tokak 157/37 plants, an alleged landrace, were studied using 30 SSR markers. Of the 30 SSR markers, only 5 were polymorphic. These polymorphic markers (BMAG606, BMAG120, GBMS50, GBMS137, and GBMS247) showed that there were only 2 types of genotype among all 60 plants analyzed. The first type of the marker profile was observed in 58 lines, and the second type in 2 lines (lines 7 and 58). No heterozygosity was observed for any marker in any line.

Morphological markers were investigated in 60 cv. Tokak 157/37 plants, and they did not show any polymorphism. All plants had long and very pubescent rachillae, well-developed sterile florets, rough awns, and leaves with anthocyanin. According to these results, CIHO 10093 and Tokak 157/37 lines had the same morphological features.

PI 470281 material

In all, 52 landrace PI 470281 plants were studied using 30 SSR markers, 23 of which were polymorphic (Figure 1). The number of alleles and the PIC value for each marker are given in Table 1.

The 30 SSR markers used on 52 landrace PI 470281 plants produced 70 alleles. The number of alleles per marker varied from 1 to 6 (mean: 2.28). GBMS117 and GBMS120 had 6 alleles; GBMS137 had 4 alleles; BMAG606, BMAG507, BMAG321, BMAG120, GBMS50, GBM1464, and GBM1055 had 3 alleles; and BMAG518, BMAG369, BMAG247, BMAG222, BMAG217, GBMS235, GBMS229, GMS166, GBMS129, GBMS66, GBMS35, HVM40, and EBMAC906 had 2 alleles. PIC values of the polymorphic markers ranged from 0.04 to 0.75. Mean PIC value of all markers was 0.28.

A dendrogram was prepared to visualize the relationships between the lines of all 3 materials (Figure 2). Five groups appeared in the dendrogram. Group 1 had the CIHO 10093 pure line, 2 types found in cv. Tokak 157/37, and line 217 of PI 470281. Group 2 had lines 56 and 228 of PI 470281, group 3 had lines 53, 200, and 212 of PI 470281, and group 4 had lines 46, 62, 64, and 218. Group 5 was the largest and had 42 PI 470281 lines. This group was divided into 3 sub-groups: sub-group A had lines 44, 58, 59, 203, 224, 214, 215, 207, 208, 229, 213, 222, and 223 of PI 470281; sub-group B had lines 42, 45, 50, 54, 57, 201, 202, 211, and 226; and sub-group C had lines 40, 43, 47, 48, 51, 60, 61, 63, 65, 67, 204, 205, 206, 209, 210, 219, 220, 221, 225, and 227. There were 46 different genotypes in the 52 lines of PI 470281. Based on the markers studied, the following lines were identical: 40, 43, and 48; 54 and 57; 201 and 202; 50 and 211; and 207 and 208. There were no heterozygous marker profiles for any marker in any plant.

Fifty-two PI 470281 lines were evaluated under field conditions for such traits as flowering time, 1000-seed weight, spike yield, and number of seeds per spike (data not shown). Variation within PI 470281 in any of these traits was not considerable. Morphological traits were investigated in 52 PI 470281 plants and polymorphism was not observed in any of these plants. All lines had long and very pubescent rachillae, well-developed sterile florets, rough awns, and leaves without anthocyanin.

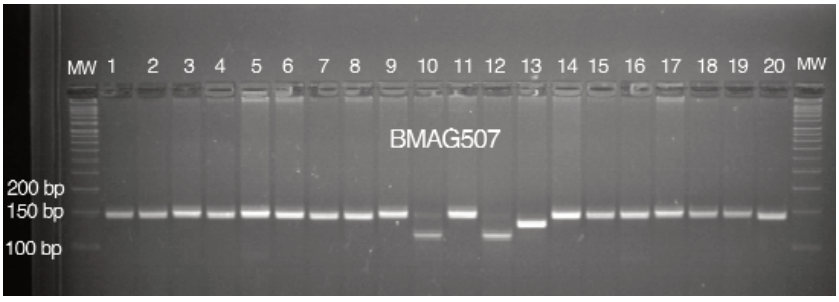

Figure 1. The SSR marker profile of 20 PI 470281 lines using the BMAG507 marker. First and last lanes show molecular weight (MW) markers. Length of bands in lanes 10 and 12 is 114 bp, lane 13 130 bp, and other lanes 146 bp.

Table 1. Some general information about the SSR markers used in the study and the results of marker analysis.

SSR Marker	Repeat type	Chromosome	Number of alleles	Annealing temperature °C	PIC
BMAG369	(CT)16	1	2	58	0.23
BMAG321	(AG)17(AC)16	1	3	58	0.60
GBMS120	(AT)35	1	6	60	0.73
GBMS129	(CA)11	1	2	55	0.33
GBMS35	(GA)12	1	2	60	0.36
GBM1464	(CAG)8N(CAG)5	1	3	55	0.37
BMAG217	(AG)19	1	2	58	0.36
BMAG507	(AG)23	1	3	53	0.18
BMAG120	(AG)15	1	3	58	0.25
GBMS66	(CA)10	2	2	60	0.20
GBMS229	(GT)10	2	2	55	0.04
GBMS247	(GT)9	2	2	60	0.20
GBMS235	(TC)12T(TC)23	2	2	55	0.04
GBMS137	(GA)40	2	4	60	0.73
BMAG518	(TC)23	2	2	58	0.44
SCSSR7759	(GCA)6(CAG)8	2	1	58	0
GBMS2	(GA)14	2	1	60	0
GBMS50	(AC)13(AG)14	3	3	55	0.47
GBMS117	(CT)9	3	6	60	0.63
GBMS166	(CA)6TCGCT(CA)9	3	2	60	0.49
BMAG606	(CT)22	3	3	55	0.75
HVM40	(GA)6(GT)4(GA)7	4	2	55	0.44
GBM1055	(CCT)6	4	3	55	0.53
EBMAC691	(CA)3CG(CA)7	4	1	55	0
GBMS114	(CT)9	4	1	60	0
EBMAC906	(GC)5GGG(GT)16	4	2	60	0.04
GBM1323	(GCC)8	4	1	55	0
HVPAZXG	(C)12(A)17	4	1	55	0
BMAG222	(AC)9(AG)17	7	2	58	0.18
GBMS77	(GT)18	7	1	60	0
Total			70		8.59
Average			2.4		2.28

Discussion

As no polymorphism was observed in the 10 CIHO 10093 plants using 30 SSR markers, it can be stated that CIHO 10093 is a pure line. SSR analysis conducted on cv. Tokak 157/37 plants, an alleged landrace cultivar (Akar et al. 1999), showed that 58 of the 60 plants studied had the same marker pattern and that the other 2 had a different marker pattern. This level of variation is very low for a landrace; therefore, Tokak 157/37 seems to have lost its landrace characteristics.

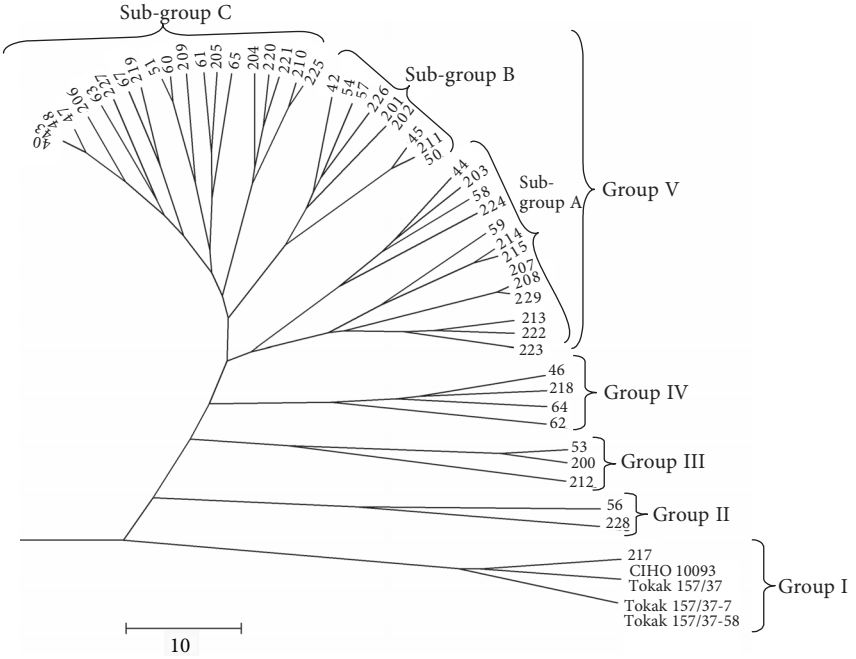

Figure 2. Radial dendrogram based on SSR marker data.

CIHO 10093 had the same marker profile as the common type found in 58 lines of Tokak 157/37. Similarly, there was no polymorphism in the lines of these 2 materials for the morphological markers studied. Thus, CIHO 10093 is the same as the common genotype of cv. Tokak 157/37.

A total of 70 alleles were obtained from 30 SSR markers used on 52 lines of PI 470281. The number of alleles per marker varied from 1 to 6 (mean: 2.28). These values seem to be small compared to previously published values; however, it should be noted that most other studies employed wild species (Struss and Plieske 1998; Matus and Hayes 2002), many different landraces (Hamza et al. 2004; Pandey et al. 2006), and many materials from different geographical regions of the world (Pillen et al. 2000; Kolodinska Brantestam et al. 2007). In contrast, the above-mentioned values obtained in the present study were for only a single landrace (PI 470281). Struss and Plieske (1998) found an average of 8.6 alleles per SSR marker in 163 barley genotypes maintained in Germany's IPK Gene Bank. When calculated, the number of alleles per genotype in our study is close to their values. Accordingly, it can be stated that genetic variation in PI 470281 is very high.

PIC values of polymorphic SSR markers for PI 470281 varied from 0.04 to 0.75 (mean: 0.28). These values were somewhat lower than those obtained in other studies; however, the mean PIC value of 0.28 obtained in the present study for a single landrace is comparable to that reported by Pillen et al. (2000), who studied 28 German cultivars and 2 wild germplasms, and obtained a mean PIC value of 0.38. These results also show the high genetic variation in PI 470281. This material deserves additional research to identify new gene alleles for agronomically important traits. The high level of genetic variation observed in marker analysis, which was not present in morphological markers and the plant characteristics, is typical of landraces (Harlan 1975). This may indicate the possibility that farmers who have been using this landrace for centuries might have performed phenotype-based mass selections within the landrace.

There were no heterozygous individuals in any of the 3 materials, indicating that there was no pollen transfer during seed multiplication. This is something expected, as landraces of self-pollinating crops are expected to be homozygous and heterogeneous.

Although considered a landrace, Tokak 157/37 has only 2 types of genotype. The common genotype of Tokak 157/37 is the same as CIHO 10093, a pure line maintained at the US NPGS with the name Tokak. PI 470281, a landrace also named Tokak, is completely different than CIHO10093 and Tokak 157/37. PI 470281 has a very high level of genetic variation, based on DNA marker analysis, which is not visible in its characters, indicating possible visual selection in the past by peasant farmers. Because of its high level of variation the agronomic and quality traits of PI 470281 should be investigated further.

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References

Akar T, Avcı M, Düşünceli F, Tosun H, Ozan N, Albustan S, Yalvaç K, Sayım İ, Özen D, Sipahi H (1999) Orta Anadolu ve geçit bölgelerinde arpa tarımının sorunları ve çözüm yolları. Hububat Sempozyumu, 77-86. 8-11 June 1999. Konya.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36: 181-186

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Graingenes. GrainGenes database Homepage. Genetic Markers. (24 July 2009). <http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker>.

Hamza S, Hamida BW, Rebai A, Harrabi M (2004) SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135: 107-118.

Harlan JR (1975) Our vanishing genetic resources. Science (Washington, D.C.) 188: 618-621.

Kolodinska Brantestam A, von Bothmer R, Dayteg C, Rashal I, Tuvesson S, Weibull J (2007) Genetic diversity changes and relationships in spring barley (Hordeum vulgare L.) germplasm of Nordic and Baltic areas as shown by SSR markers. Genet Resour Crop Ev 54: 749-758.

Maestri E, Malcevschi A, Massari A, Marmiroli N (2002) Genomic analysis of cultivated barley (*Hordeum vulgare*) using sequence-tagged molecular markers. Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes, and simple-sequence repeats (SSRs). Mol Genet Genomics 267: 186-201.

Matus IA, Hayes PM (2002) Genetic diversity in three groups of barley germplasms assessed by simple sequence repeats. Genome 45: 1095-1106.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Pandey M, Wagner C, Friedt W, Ordon F (2006) Genetic relatedness and population differentiation of Himalayan hulles barley (*Hordeum Vulgare* L.) landraces inferred with SSR. Theor Appl Genet 113: 715-729.

Parzies HK, Spoor W, Ennos RA (2000) Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare* conserved for different lengths of time in ex situ gene banks. Heredity 84: 476-486.

Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Förster J, Leon J (2000) Mapping new EMBL-derived barley SSRs and their use in differentiating German barley cultivars. Theor Appl Genet 101: 652-660.

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149: 2007-2023.

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97: 1-2308-315.

Varshney RK, Grosse I, Hahnel 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. Theor Appl Genet 113: 239-250.

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23: 48-55.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada (program available from: http://www.ualberta.ca/fyeh/).