Hereditas 135: 145–151 (2001)

# Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.)

RAJA KOTA, RAJEEV KUMAR VARSHNEY, THOMAS THIEL, KLAUS JOHANNES DEHMER and ANDREAS GRANER

*Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*

**Kota, R., Varshney, R. K., Thiel, T., Dehmer, K. J. and Graner, A. 2001.** Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.).—*Hereditas 135*: 145–151. Lund, Sweden. ISSN 0018-0661.

The progress of genome sequencing projects of model plants like barley, combined with the recent advances of high throughput assays, has provided a wealth of sequence information. This information is being employed to develop a high density transcript map of barley (*Hordeum vulgare* L.). To achieve this goal, the available EST database is being used as a resource for the development of novel microsatellite (SSR) and single nucleotide polymorphism (SNP) markers. So far, a total of 692 microsatellites representing different di-, tri- and tetra-nucleotide repeats were identified from a set of 19,000 EST sequences. Non-redundant SSRs have been used for mapping and so far 76 microsatellite loci were mapped. In addition to the 180 SNP primer pairs, which were designed to target specific ESTs, 72 were polymorphic among the seven genotypes examined here. Of these, 60 SNPs have been mapped applying a denaturing HPLC approach. To examine the potential of the EST-derived markers for pedigree studies, EST-derived SSRs (75 loci) and SNPs (72 loci) were used to fingerprint a set of seven genotypes. The results show that although both marker types yielded similar groupings, a larger data set of both SSRs and SNPs is necessary to obtain stable clusters in unrelated germplasm.

*Raja Kota, Institute of Plant Genetics and Crop Plant Research (IPK), Correnstr. 3, 06466 Gatersleben, Germany.* E-mail: kota@ipk-gatersleben.de

The analysis of DNA sequence variation is of major importance in genetic studies like genome mapping, gene tagging and pedigree analysis. In this context, molecular markers represent a major tool and have been extensively used for this purpose in barley. Several approaches have been pursued to detect sequence polymorphism and preparation of molecular maps including RFLP (restriction fragment length polymorphism; e.g. GRANER et al. 1991; KLEINHOFS et al. 1993), RAPD (random amplified polymorphic DNA; e.g. WEYEN et al. 1996), SSR (simple sequence repeat; e.g. PILLEN et al. 2000; RAMSAY et al. 2000) and AFLP (amplified fragment length polymorphism; e.g. WAUGH et al. 1997). Among the different available marker systems mentioned above, microsatellites are widely considered to be the markers of choice as RFLPs are relatively labour intensive and time consuming, AFLP patterns are sometimes difficult to compare, and RAPDs frequently suffer from a lack of reproducibility.

Microsatellites or simple sequence repeats (SSRs) are arrays of short motifs of 1–6 base pairs in length and hypervariable in nature. The advantages of using microsatellite markers in plant genetics and molecular breeding are their multi-allelic nature, co-dominant inheritance, ease of detection by PCR, relative abundance, extensive genome coverage and requirement of only a small amount of sample DNA (for details see POWELL et al. 1996; GUPTA and VARSHNEY 2000). Although development of these markers is time consuming and expensive, once produced, they are simple to use and extremely reliable.

In recent times, a new class of molecular markers called SNPs (single nucleotide polymorphisms) has been available which are based on single base changes within the genome. SNPs are genetic markers, which are bi-allelic in nature, highly abundant and less prone to mutations than SSRs (GIORDANO et al. 1999). In addition, many assays have been developed to type SNPs in an automated fashion and many yield simple positive or negative results that can be interpreted easily (WANG et al. 1998). Among these assays, denaturing high performance liquid chromatography (DHPLC) has significantly reduced the processing time of detection and analysis of SNPs (OEFNER and UNDERHILL 1998; GIORDANO et al. 1999). This technique represents a highly sensitive and fully automated assay for SNP analysis, which is based on an ion-pair reversed-phase high performance liquid chromatography method (KUKLIN et al. 1997/1998).

The development of these EST sequencing projects has resulted in a wealth of sequence information, thus allowing the possibility for detecting and genotyping them. Marker types such as SSRs and SNPs can be searched in these EST databases and employed for designing locus-specific primers. In the past, development of SSR and SNP markers has been expensive, but now EST-derived SSRs and SNPs are a free by-product of the currently expanding EST data-

bases. These SSRs and SNPs are obviously limited to those species for which sufficient number of ESTs from a number of genotypes of a crop exists in the database. To date, this specific approach has been used for rice (MIYAO et al. 1996; CHO et al. 2000) and grapes (SCOTT et al. 2000). Also, generation and mapping of such EST-derived SSRs and SNPs are currently in progress in some important cereal species like wheat (EUJAYL et al. 2000; SOMMERS et al. 2001). EST-derived SSRs have some intrinsic advantages over genomic SSRs, since they are quickly obtained by electronic sorting, are unbiased in repeat type, are present in gene rich regions of the genome, and are still abundant (SCOTT 2001). The usefulness of these EST-derived SSRs and SNPs also lies in their expected transferability, since they are based on the conserved coding region of the genome. In addition to this, the identification of SSRs and SNPs from EST databases is a relatively less costly approach than the development of genomic SSRs.

ESTs provide valuable but incomplete information. However, because they represent expressed genomic regions, ESTs are thought to identify the parts of the genome with the most biological significance. Based on the DNA sequence information obtained from the barley EST database (B-EST; http://pgrc.ipk-gatersleben.de), we have investigated the usefulness of ESTs as a resource to develop and map a comprehensive set of novel markers for the barley genome.

Barley (*Hordeum vulgare* L.) is one of the most important crop species in the world crop production and is cultivated in all temperate areas (BOTHMER et al. 1995). The barley genome is well characterised with respect to classical genetics and cytogenetics, and in many regards acts as a model crop for mapping studies in cereals. The current study described here was conducted towards developing a high-density transcript map of barley. Since SNPs represent an attractive marker system for systematic fingerprinting, we have compared and performed parallel pedigree analyses using SSR and SNP markers to explore the potential of the latter for pedigree analysis in barley.

## MATERIALS AND METHODS

### *Plant materials*

In the present study, a set of seven barley (*Hordeum vulgare* L.) genotypes namely Igri, Franka, Steptoe, Morex, Oregon Wolfe Rec, Oregon Wolfe Dom and Barke was employed. We used these genotypes as they have been used in the development of three different mapping populations which are: a "Igri" × "Franka", "Steptoe" × "Morex", and "Oregon Wolfe Dom" × "Oregon Wolfe Rec", respectively. In addition to these, "Barke" was also used as a reference genotype because this spring barley cultivar was employed in our EST sequencing programme.

### *SSR discovery and analysis*

For development of EST-derived SSR markers, the IPK barley EST database (B-EST) consisting of 13,109 entries and a new set of about 6000 ESTs were searched for all possible dimeric, trimeric and tetrameric microsatellites with the help of a PERL5 script (Thiel et al., unpubl.). SSR-containing sequences were analysed using the stackPACK (version 2.1) software package (Electric Genetics, Cape Town, South Africa), to define a non-redundant set of ESTs. For marker development, primers were designed using the Primer3 software (Whitehead Institute, Cambridge, MA, USA).

PCR was done in 10 µl reactions containing 20 ng genomic DNA, 1 × PCR buffer (including 1.5 mM $MgCl_2$), 0.2 mM dNTPs, 2.5 pmol of each primer and 0.25 U *Taq* DNA Polymerase (Qiagen, Hilden, Germany). For all microsatellites, the following touch down PCR profile was used: 3 min at 94°C; 10 cycles of 30 sec at 94°C, 30 sec at 60°C minus 0.5°C/cycle, 30 sec at 72°C; 25 cycles of 30 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C; and 5 min at 72°C for final extension. PCR products were separated on 10 % denaturing polyacrylamide gels (7 M urea) and visualized by silver staining.

### *SNP discovery and DHPLC analysis*

To perform the PCR, primers were designed to amplify ESTs derived from cDNA libraries constructed using *H. vulgare* cv. Barke and which are available on the IPK barley EST database. All primers were designed using the software "Primer Express" (Applied Biosystems). PCR was done in 20 µl reactions containing 25 ng genomic DNA, 1 × PCR buffer (including 1.5 mM $MgCl_2$), 0.25 mM dNTPs, 0.5 µM of each primer and 1.0 U of *Taq* DNA polymerase (Qiagen). The touch down PCR cycling profile included an initial denaturing step at 94°C for 2 min followed by a profile of: 1 cycle of 30 sec at 94°C, 1 min at 65°C and 1 min at 72°C followed, by 9 cycles over which the annealing temperature was decreased by 1°C per cycle, followed by 40 cycles of 30 sec at 94°C, 1 min at 56°C and 1 min at 72°C. DHPLC was carried out as described in KOTA et al. (2001).

To identify SNPs, PCR products amplified from genomic DNA templates of the parental genotypes were sequenced in both directions on an ABI 377XL automated sequencer using big dye-terminator chemistry (Applied Biosystems). DNA sequence data was checked for sequencing errors using the "Se-

quencher" software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were aligned using GCG pileup program [version 7.0] (GRIBSKOV et al. 1984) and polymorphisms between the parents were identified manually.

*Cladistic analysis*

A total of 75 SSR loci and 72 SNP loci were evaluated using the PAUP* software (version 4.0b8; SWOFFORD 1998). The "total character difference" was used as distance measure for the construction of UPGMA (Unweighted Pair Group Method Arithmetic Average) phenograms. Bootstrap analyses with 5000 replicates were performed to measure the confidence level of the obtained clusters.

## RESULTS AND DISCUSSION

*SSR analysis*

EST mapping in recent times has gained momentum, especially with the wealth of sequence information that is being generated in various EST sequencing programs. A data set of around 19,000 ESTs was employed for searching the SSRs (unpubl., MICHALEK et al. 2001). We developed a Perl script to search for all possible dimeric (minimum of six repeats), trimeric and tetrameric microsatellites (minimum for both of five unit repeats). By using these criteria, we found a total of 692 microsatellites (3.6 %) including 188 di-, 463 tri- and 41 tetra-nucleotide repeats respectively. Thus in this dataset, the frequency of di-, tri- and tetra-nucleotide repeats is 27.2 %, 66.9 % and 5.9 %, respectively. The overall frequency of microsatellites obtained (3.6 %) from barley ESTs in the present study is in agreement with earlier studies conducted in grape (SCOTT et al. 2000), *Arabidopsis* (CARDLE et al. 2000) and sugarcane (CORDEIRO et al. 2001) where the microsatellites were obtained at frequencies of 2.5 %, 3 % and 2.8 %, respectively. Further, the relative frequencies of di-, tri-, and tetra-nucleotide repeats in our data set is also in accordance with the frequencies of that observed in rice, maize, soybean, tomato and cotton, where tri-nucleotide repeats were found to be abundant in EST sequences (CARDLE et al. 2000).

Out of these 692 microsatellites, primer pairs could only be designed for 532 of them. For the remaining 23 % microsatellites (160), primer pairs could not be designed for one of the following reasons: (1) microsatellite sequences were too short, (2) microsatellites were too close to the cloning site of EST, or (3) the flanking sequences were not unique. In an earlier study on barley microsatellites, RAMSAY et al. (2000) could also design the primer pairs for only 48 % microsatellites.

Prior to the synthesis of primers, all the 532 microsatellites were subjected to redundancy analysis. By using a subset of these primer pairs, we found that around 66 % are functional. The remaining primer pairs (34 %) failed to amplify products. This could be due one or both primers of the primer pairs being designed across exon/intron splice sites preventing genomic DNA to be amplified (CORDEIRO et al. 2001). Of these functional primer pairs, about 78 % of them gave the product of expected size, while the remaining 22 % primer pairs yielded an amplicon which was bigger than expected size. The formation of products of unexpected size is mainly due to the presence of introns which confirms the conjecture that failure of amplification may be partly due to the presence of such sequences. In the present study, we have used the data of 75 polymorphic microsatellite loci on a set of 7 barley genotypes to test their potential for pedigree analysis and for comparing with that of SNPs (Table 1).

*SNP analysis*

In the ongoing mapping projects in our group, we are mapping ESTs employing RFLPs and SSRs towards the generation of a high-density transcript map of barley. Although RFLP and SSR assays have been successful in mapping ESTs, approximately 38 % of the EST-RFLPs and 58 % of the EST-SSRs were monomorphic in a given set of mapping populations (unpublished data). To place these ESTs on the barley map, we are trying to capitalise on the presence of SNPs in these sequences.

Table 1. *Summary of results generated from the SSR and SNP studies. Basic information includes the number of respective markers mapped to various chromosomes in the three mapping populations of barley (primer sequences for SSRs and SNPs are available on request)*

| Chromosome | 1H | 2H | 3H | 4H | 5H | 6H | 7H | Unmapped | Total |
|---|---|---|---|---|---|---|---|---|---|
| Marker type | | | | | | | | | |
| SSR | 5 | 4 | 4 | 5 | 2 | 1 | 4 | 50 | 75 |
| SNP | 7 | 12 | 13 | 7 | 8 | 6 | 7 | 12 | 72 |
| Total | 12 | 16 | 17 | 12 | 10 | 7 | 11 | 67 | 147 |

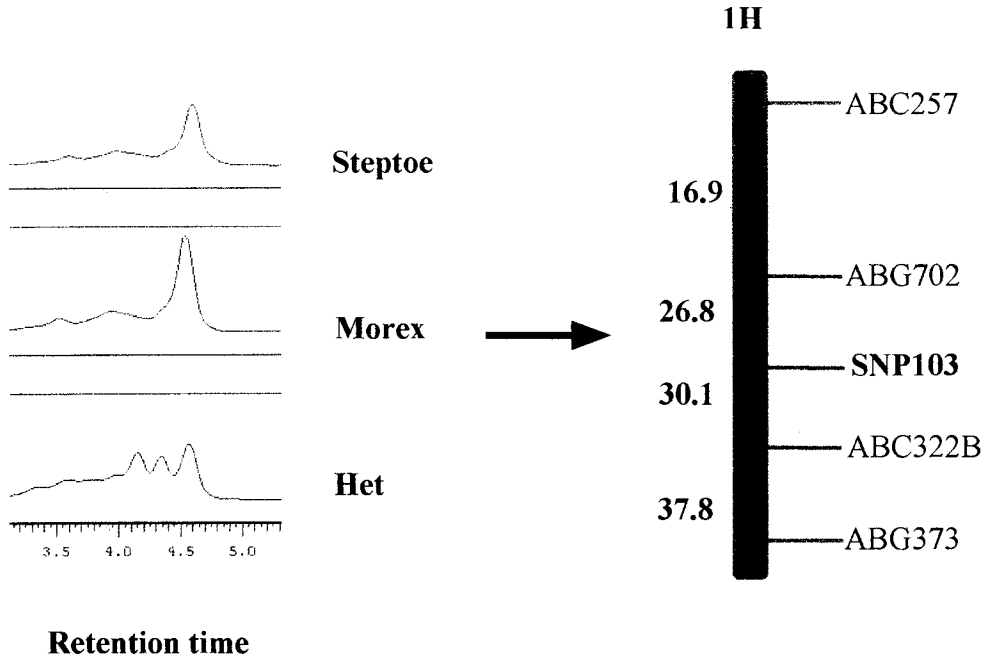


**Fig. 1.** Elution profile and a partial map of barley chromosome 1H displaying the genetic position of marker SNP103 from barley in the "Steptoe" × "Morex" DH population. "Het" represents a mixture of PCR products from "Steptoe" and "Morex" in equimolar ratio.

However, before using a new marker type in large-scale genomic projects and in marker-assisted selection, it is relatively important to obtain the frequency of polymorphisms and the capacity to detect and analyse these marker types with relative ease. In this context, from the data generated so far among the seven genotypes, the frequency of SNP occurrence in barley was estimated to be one every 240 nucleotides. This result was calculated based on 52,140 bp of DNA sequence from each genotype analysed. Similar studies have been conducted in crop species such as *Beta vulgaris* and *Zea mays*, where the frequency of SNP occurrence was estimated to be one in every 130 and 104 nucleotides respectively (SCHNEIDER et al. 2001; TANAILLON et al. 2001). As expected, the frequency of SNPs in an inbreeding species such as barley is lower than in the outbreeding species mentioned before. Nevertheless, the present data confirm that the frequency of SNP occurrence in barley is sufficient to make them appropriate markers for any kind of genetic studies.

So far, 180 SNP primer pairs have been designed which primarily target the 3′untranslated regions of the genome as, they have been proven be more polymorphic and make good candidates for developing SNP markers in barley. Even though stringent parameters were set for primer design, approximately 9 % of primer pairs failed to amplify any product, probably for similar reasons as given above. The decreased frequency of failure may be connected to a smaller size of the amplicons used for SNP analysis. In addition, approximately 11 % of the primer pairs amplified multiple products, which were discarded at this stage from further analysis because of problems anticipated for DNA sequencing and DHPLC analysis.

The screening of SNPs was performed by DHPLC, based on the differential retention of homo- and heteroduplex DNA molecules under conditions of partial denaturation (Fig. 1). In the present work, optimal temperatures for discrimination were determined by employing the algorithm available with the WAVEmaker software (Transgenomics., Omaha, NE, USA). Although, the algorithms are fairly accurate, we have been analysing our samples in 1–3°C increments over the range of predicted melting temperatures to achieve greater sensitivity (KOTA et al. 2001). In cases where the sequence of the PCR fragment is not known, samples will have to be run at several temperatures making it a time-consuming process (WAGNER et al. 1999). Considering this factor, we decided to sequence the PCR fragments generated from the parental genotypes, as this method in our experience has proven to be a faster and more efficient way to identify potential SNPs rather than trying to identify them directly on the DHPLC. Compared to most primer extension procedures applied to detect SNPs, a major advantage of the procedure

described in this study is the savings in labour costs associated with purifying PCR products, setting up and loading of sequencing reactions and reading the sequencing gels. This holds true in our case where only a few sequencing reactions were necessary to reveal the chemical nature of a mutation.

Employing the SNP strategy, we have been able to increase the efficiency of mapping those ESTs that were monomorphic at the RFLP and SSR level respectively (unpublished data). Therefore, the SNP analysis represents a sensitive tool to maximise the number of ESTs that can be mapped, as it will be required for the construction of a comprehensive transcript map of barley. Employing the above process, we identified 72 SNPs among the three mapping populations, of which 60 have been mapped so far (Table 1). While mapping SNPs is a major objective of this work, pedigree analysis of the seven genotypes was undertaken to compare the potential usefulness of SNPs to that of SSRs, which have proven well suited for phenetic analyses.

*Cladistic analysis*

The phenogram obtained on the basis of 75 microsatellite loci differentiates the genotypes in three major groups (Fig. 2A). On the basis of the 50 % consensus rule bootstrap values of the microsatellite data, four of the six nodes are supported. These are: (i) 97 % for Igri and Franka; (ii) 59 % for Steptoe, Morex and Barke; (iii) 80 % for Rec and Dom; and (iv) 69 % for separating Igri/Franka from Steptoe/Morex/Barke. The cladogram obtained from the SNP data reflects two clusters of two or three varieties, respectively, as well as two ungrouped varieties

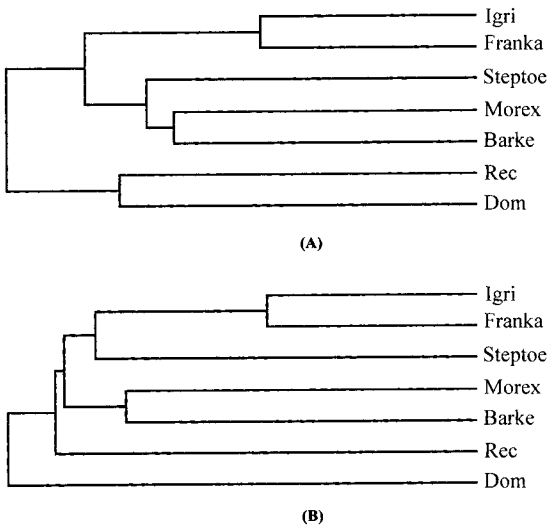


**Fig. 2.** UPGMA phenograms showing the genetic diversity among the seven barley genotypes using (A) SSR and (B) SNP data, respectively.

(Barke and Dom) (Fig. 2B). Here, the 50 % consensus rule bootstrap values support only three nodes. These are: (i) 97 % for Igri/Franka separated from the rest; (ii) 55 % for separating Dom from all other varieties; and (iii) 51 % for separating Morex and Rec from Barke, Steptoe and Igri/Franka, respectively.

Regarding the mapping parents, Igri and Franka form a separate sub-cluster displaying the lowest level of polymorphism (simple matching coefficient 0.73 or 0.75 for SSR or SNP, respectively), followed by Steptoe and Morex (0.59/0.58), while Dom and Rec are the most polymorphic mapping parents (0.56/0.44). These results clearly correspond to the observations made in several mapping projects (GRANER et al. 1991; KLEINHOFS et al. 1993; COSTA et al. 2001).

With respect to pedigrees, each of the above marker assays could separate Igri and Franka (both of German origin), from the other varieties. Furthermore, the SNP data manage to differentiate Steptoe more clearly from Morex, thus distinguishing the North African germplasm pool from the Asian Manchuria one. SNPs also do cluster Rec and Dom separately, which is in accordance with the findings from KORTE et al. (http://wheat.pw.usda.gov/gg-pages/SSR/Korte) while the SSRs group them together. Concerning the confidence levels of the cladograms obtained by the two marker assays (and three data sets, see Materials and Methods), however, SSRs prove to be superior to SNPs when using comparable amounts of data (bootstrap values not shown). This might be due to the high degree of informativeness of SSRs when compared to SNPs.

This would indicate that more SNP loci have to be scored to achieve a similarly reliable result with that obtained from the SSR loci in a given set of genotypes. Nevertheless, even 75 SSR markers do not suffice for obtaining very well supported bootstrap values. The even lower reliability of the 115 individual SNP mutations (data not shown) in this respect is contradictory to the observations of a recent study (KANAZIN et al. 2001), who found no major differences between the SNP mutations and SNP loci. This difference may be accounted for by the different sets of plant material analysed in the two studies. While KANAZIN et al. (2001) examined germplasm groups representing three distinct genepools, the barley accessions used in this study are probably more diverse. Only Igri and Franka belong to the genepool of European winter barley germplasm. These winter barleys are characterised by a set of common ancestors, which are genetically distinct from European spring barleys such as cv. Barke (MELCHINGER et al. 1994). Indeed, the dendrograms constructed with both marker types group Igri together with Franka and place Barke separately. The remaining accessions

analysed in this study initially were selected as mapping parents based on their diverse origins: Steptoe and Morex trace back to North African and Central Asian germplasm. The Oregon Wolfe barleys have shown to be the most polymorphic of the three mapping populations analysed. Their pedigree remains obscure, since they arose from crosses between various unrelated marker stocks. Since both the SSRs and the SNPs were derived from coding sequences and their distribution across the genome seems to be at random, we did not expect a bias arising from a differential sampling of the genome (Table 1). The analysis of additional markers will reveal if it is possible to obtain a congruent phenogram with both marker types.

*Conclusion*

From the results obtained so far, we demonstrate that SSRs and SNPs are successfully being generated from ESTs. These markers are being mapped with an ultimate objective to develop a high-density transcript map of barley. The EST map will facilitate the alignment of existing linkage maps in barley and would permit the identification of ESTs that are associated with traits of interest. Moreover, data generated from these studies could be employed for syntenic studies with rice and other related Triticeae species. Results from the two marker assays for pedigree analysis yielded similar but not identical outcomes. In this context, the SNP cladogram was less supported by bootstrap values than that obtained from the SSR data. Based on the results obtained from the present study, SNPs might be slightly inferior to SSRs in phenetic analyses. Nevertheless, for both marker types, larger data sets need to be generated to obtain reliable results.

## ACKNOWLEDGEMENTS

We are grateful to Patrick Hayes for providing the DH lines of barley mapping populations "Steptoe" × "Morex" and "Oregon Wolfe Dom" × "Oregon Wolfe Rec". We thank Winfriede Weschke and Elena Potokina for providing cDNA libraries for this study. Technical assistance of Ulrike Beier and Christine Künzel is gratefully acknowledged. This work was funded by the BMBF (grants 0312271A and 0312278C).

## REFERENCES

Bothmer R von, Jacobsen N, Baden C, Jorgensen RB and Linde-Laursen I, (1995). An ecogeographical study of the genus Hordeum. Int. Plant Genet. Resour. Rome, Italy.

Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D and Waugh R, (2000). Computational and experimental characterisation of physically clustered simple sequence repeats in plants. Genetics 156: 847–854.

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N and Cartinhour S, (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (Oryza sativa L.). Theor. Appl. Genet. 100: 713–722.

Cordeiro GM, Casu R, McIntyre CL, Manners JM and Henry RJ, (2001). Microsatellite markers from sugarcane (Saccharum spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci. 160: 1115–1123.

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Lee M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI and Wolfe RI, (2001). Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor. Appl. Genet. 103: 415–424.

Eujayl I, Baum M, Sorrells M, Wolters P and Powell W, (2000). Use of EST-derived microsatellites to estimate genetic diversity in commercial durum wheat. In: Tenth International Public Workshop, 14–16 June, 2000. University of Delaware, Newark, Delaware, USA.

Graner A, Jahoor A, Schondelmaier H, Siedler K, Pillen K, Wenzel G and Herrmann RG, (1991). Construction of an RFLP map of barley. Theor. Appl. Genet. 83: 250–256.

Giordano M, Oefner PJ, Underhill PA, Luca Cavalli Sforza L, Tosi R and Richiardi PM, (1999). Identification by denaturing high-performance liquid chromatography of numerous polymorphisms in a candidate region for multiple sclerosis susceptibility. Genomics 56: 247–253.

Gribskov M, Devereux J and Burgess RR, (1984). The codon preference plot: graphic analysis of protein coding sequences and prediction of gene expression. Nucl. Acids Res. 12: 539–549.

Gupta PK and Varshney RK, (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163–185.

Kanazin V, Talbert H, See D, DeCamp P, Nevo E and Blake T, (2001). Discovery and assay of single nucleotide polymorphism in barley (Hordeum vulgare). Plant Mol. Biol. (In press)

Kleinhofs A, Kilian A, Saghai-Maroof M, Biyashev R, Hayes P, Chen F, Lapitan N, Fenwick A, Blake T, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp S, Liu B, Sorrells M, Heun M, Franckowiak J, Hoffman D, Skadsen R and Steffenson B, (1993). A molecular isozyme and morphological map of the barley (Hordeum vulgare) genome. Theor. Appl. Genet. 86: 705–712.

Kota R, Wolf M, Michalek W and Graner A, (2001). Application of DHPLC for mapping of single nucleotide polymorphisms (SNPs) in barley (Hordeum vulgare L.). Genome 44: 523–528.

Kuklin A, Munson K, Gjerde D, Haefele R and Taylor P, (1997/1998). Detection of single nucleotide polymorphisms with the WAVE DNA fragment analysis system. Genet. Test. 1: 201–206.

Melchinger AE, Graner A, Singh M and Messmer M, (1994). Relationships among European barley germplasm: I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci. 34: 191–1199.

Michalek W, Weschke W, Pleissner K-P and Graner A, (2001). EST analysis in barley defines a unigene set comprising 4,000 genes. Theor. Appl. Genet. (in press)

Miyao A, Zhong HS, Monna L, Yano M, Yamamoto K, Havukkala I, Minobe Y and Sasaki T, (1996). Characterisation and genetic mapping of simple sequence repeats in the rice genome. DNA Res. 3: 233–238.

Oefner PJ and Underhill PA, (1998). DNA mutation detection using denaturing high performance liquid chromatography (DHPLC). In: Current protocols in human genetics. Wiley and Sons, USA.

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

Powell W, Machray GC and Provan J, (1996). Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1: 215–222.

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

Schneider K, Weisshaar B, Borchardt DC and Salamini F, (2001). SNP frequency and allelic haplotype of Beta vulgaris expressed genes. Mol. Breed. 8: 63–74.

Scott KD, (2001). Microsatellite derived from ESTs, and their comparison with those derived by other methods. In: Plant genotyping: the DNA fingerprinting of plants (ed. RJ Henry). CABI Publishing, Oxon, UK, p. 225–237.

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS and Henry RJ, (2000). Analysis of SSRs derived from grape ESTs. Theor. Appl. Genet. 100: 723–726.

Sommers DJ, Kirkpatrick R, Moniwa M, Walsh A and Gustafson JP, (2001). Mapping wheat ESTs by haplotype specific SNPs. In: XVIth Eucarpia Congress, Plant breeding: sustaining the future, Abstract P1.25, 10th-14th Sept. 2001, Edinburgh, UK.

Swofford DL, (1998). PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

Tanaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF and Gaut BS, (2001). Patterns of DNA sequence polymorphism along chromosome 1 of maize (Zea mays ssp. mays L.). Proc. Natl. Acad. Sci. USA 98: 9161–9166.

Wagner T, Stoppa-Lyonnet D, Fleischmann E, Muhr D, Pagès S, Sandberg T, Caux V, Moeslinger R, Langbauer G, Borg A and Oefner P, (1999). Denaturing high performance liquid chromatography (DHPLC) detects reliably BRCA1 and BRCA2 mutations. Genomics 62: 369–376.

Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M and Lander ES, (1998). Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science 280: 1077–1082.

Waugh R, Bonar N, Baird E, Thomas B, Graner A, Hayes P and Powell W, (1997). Homology of AFLP products in three mapping populations of barley. Mol. Gen. Genet. 255: 311–321.

Weyen J, Bauer E, Graner A, Friedt W and Ordon F, (1996). RAPD-mapping of the distal portion of chromosome 3 of barley, including the BAMMV/BAYMV resistance gene ym4. Plant Breed. 115: 285–287.