NOTE / NOTE

Genotyping single nucleotide polymorphisms in barley by tetra-primer ARMS–PCR

E. Chiapparino, D. Lee, and P. Donini

Abstract: Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA polymorphism. These polymorphisms can be used in plants as simple genetic markers for many breeding applications, for population studies, and for germplasm fingerprinting. The great increase in the available DNA sequences in the databases has made it possible to identify SNPs by "database mining", and the single most important factor preventing their widespread use appears to be the genotyping cost. Many genotyping platforms rely on the use of sophisticated, automated equipment coupled to costly chemistry and detection systems. A simple and economical method involving a single PCR is reported here for barley SNP genotyping. Using the tetra-primer ARMS–PCR procedure, we have been able to assay unambiguously five SNPs in a set of 132 varieties of cultivated barley. The results show the reliability of this technique and its potential for use in low- to moderate-throughput situations; the association of agronomically important traits is discussed.

Key words: single nucleotide polymorphisms (SNPs), genotyping, barley, tetra-primers ARMS–PCR.

Résumé : Les polymorphismes mononucléotidiques (SNP) constituent la forme la plus abondante de polymorphismes au sein de l'ADN. Ces polymorphismes peuvent être exploités chez les plantes comme marqueurs génétiques simples en sélection, pour des études de populations ou pour la production d'empreintes génétiques. Le grand accroissement du nombre de séquences d'ADN disponibles dans les banques de séquences a rendu possible l'identification de SNP par interrogation des banques et le principal facteur limitant leur utilisation à grande échelle semble être le coût du génotypage. Plusieurs plates-formes de génotypage nécessitent le recours à des appareillages sophistiqués et automatisés en combinaison avec des chimies et des systèmes de détection coûteux. Une méthode simple et économique faisant appel à une seule réaction PCR est rapportée ici pour le génotypage chez l'orge. À l'aide de l'approche ARMS–PCR à quatre amorces, les auteurs ont déterminé de façon claire le génotype de 132 variétés de l'orge cultivée pour cinq SNP. Les résultats montrent que cette technique est reproductible et illustrent son potentiel pour des applications où un débit faible à modéré est requis. L'association avec des caractères agronomiques est discutée par les auteurs.

Mots clés : polymorphismes mononucléotidiques (SNP), génotypage, orge, ARMS–PCR à quatre amorces.

[Traduit par la Rédaction]

Introduction

DNA sequence differences are the basic requirement for the study of molecular genetics. The way that these polymorphisms have been assayed reflects the technology available. The hybridization technique RFLP (Southern 1975) was superseded by amplification methodologies after the invention of PCR (Saiki et al. 1988); RAPDs (Williams et al. 1990) and AFLP (Vos et al. 1995) are able to produce many markers in a single PCR without prior sequence knowledge of the genomes of interest. These techniques are powerful and able to amplify many loci within a single reaction and are useful for assessing genetic diversity (Ridout and Donini 1999) or in the creation of genetic maps (Waugh et al. 1997); these markers are, however, often anonymous.

More recently, genetic analyses of single nucleotide polymorphisms (SNPs) are gaining interest fueled by the ever-increasing sequence data available that have revealed their abundance. For instance, Tenaillon et al. (2001) found an average of one SNP every 10^4 bp in maize between two randomly sampled sequences; similar results have been obtained surveying sequence polymorphisms in eight lines of

Received 3 August 2003. Accepted 4 November 2003. Published on the NRC Research Press Web site at http://genome.nrc.ca on 16 March 2004.

Corresponding Editor: T. Schwarzacher.

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 doi: 10.1139/G03-130

Beta vulgaris (Schneider et al. 2001). This abundance allows the construction of high-density genetic maps offering great potential to detect associations between allelic forms of a gene and phenotypes (Rafalski 2002). In addition, the dramatic increase in the number of DNA sequences submitted to the databases has made it possible to identify many SNPs for several crops without the need for sequencing (SNP e-mining). The availability of expressed sequenced tag (EST) databases makes it possible to target the polymorphisms to functional regions of the genomes and even to specific genes (Kota et al. 2001; Useche et al. 2001).

Many methods have been developed for SNP genotyping (reviewed in Landegren et al. (1998) and Bhattramakki and Rafalski (2001)). These tests detect single-nucleotide variations and usually rely on expensive DNA sequencing equipment for genotyping, as for example in performing single-base extension or pyrosequencing (Ronaghi et al. 1998). Others are based on hybridization assays that require radioactivity or reporter molecules such as the TaqMan system (PE Biosystem, Warrington, U.K.). These methods are developed for high throughput with cost being a secondary issue. The expense and practicality of the above solutions have so far limited the uptake of this class of DNA markers.

A simple and economical method involving a single PCR is reported here for barley SNP genotyping. Using the tetra-primer ARMS–PCR procedure (Ye et al. 2001), we have been able to assay unambiguously five SNPs in a set of 132 varieties of cultivated barley. The method proved to be reliable, inexpensive, and easy to use.

Materials and methods

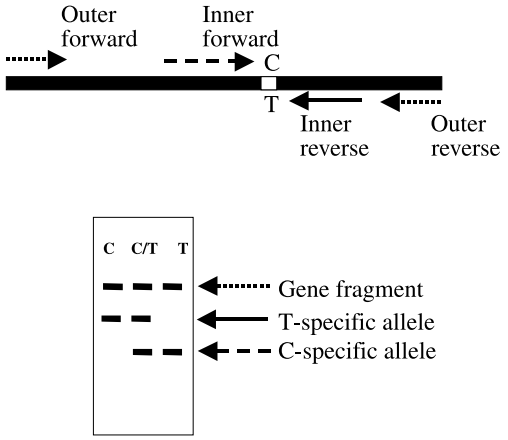
SNP information and plant material

SNP information on and sequences for nine barley loci (MWG2062, ABC465, MWG2218, ABG601, MWG502, ABG704, MWG2029, ABC156, and MWG801) were obtained from Drs. Tom Blake and Vladimir Kanazin (Montana State University; http://hordeum.oscs.montana.edu/locus/index.html) who also kindly provided DNA of the five barley varieties Baronesse, Karl, Lewis, Morex, and Steptoe previously surveyed for SNP discovery (Kanazin et al. 2002). In addition to these five barley varieties, DNA from a further 132 spring and winter barley cultivars, extracted from bulked seeds, was tested (Donini et al. 1998).

Primer design and SNP genotyping

The tetra-primer ARMS–PCR procedure (Ye et al. 2001) was used to genotype the barley varieties at each of nine SNP loci. The method employs four primers to amplify a larger fragment from DNA containing the SNP and amplicons representing each of the two allelic forms (Fig. 1). Primers can be designed to amplify fragments of differing sizes for each allele band in order for them to be easily resolved using agarose gel electrophoresis. To increase the specificity of the reaction, a mismatch is introduced at the 3′ end of each of the two allele-specific primers. Primers were designed using the primer design computer program made accessible by Ye et al. (2001) (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The primers were designed by limiting the fragment sizes to the range of

Fig. 1. Diagrammatic representation of SNP identification using the technique of tetra-primer ARMS–PCR. Four primers are used: the two outer primers amplify a fragment of the gene that contains an SNP (white box). The inner primers are designed to amplify the two allelic states (e.g., in a C→T transition, one primer will amplify the C allele and the other the T allele).



150–400 bp and the ratio of the allelic bands to 1.2–1.5. Default settings were used for other parameters. PCR was performed in a total volume of 10 μL containing 30 ng of template DNA, 10 pmol of each inner primer, 1 pmol of each outer primer, 200 μM dNTPs, 2.5 mM $MgCl_2$, 1× buffer, and 1.5 U of *Taq* polymerase (Biogene, Kimbolton, Cambs., U.K.). PCR amplifications for MWG502 and ABC156 were performed with a simple profile: 94 °C for 2 min, 35 cycles of 1 min at 94 °C, 1 min at 63 and 58 °C, respectively, and 1 min at 72 °C, ending with 2 min at 72 °C to complete extension. The other loci were amplified with the following touchdown profiles: 94 °C for 2 min, 35 cycles of 1 min at 94 °C, 1 min of annealing, and 1 min of extension at 72 °C, ending with 2 min at 72 °C. Annealing was 72 or 68 °C for the first cycle (see Table 1), decreasing by 1 °C until the annealing temperature indicated in Table 1 was reached and then continuing at that temperature in the annealing step for the remaining cycles. The PCR products were mixed with 10 μL of loading buffer (10% Ficoll, 100 mM EDTA, pH 8, 0.05% (w/v) Orange G) and 12 μL was electrophoresed in a 1.5% agarose gel and stained with ethidium bromide.

Validation of genotyping scores by tetra-primer ARMS–PCR

To validate the accuracy of the tetra-primer ARMS–PCR method, primer sets were tested on the five barley varieties Baronesse, Karl, Lewis, Steptoe, and Morex that were utilized for SNP discovery by Kanazin et al. (2002). Nine loci (MWG2062, ABC465, MWG2218, ABG601, MWG502 ABG704, MWG2029, ABC156, and MWG801) were chosen from the 54 available because they permitted the design of suitable primer sets for tetra-primer ARMS–PCR SNP detection in agarose gels. Table 1 shows the nature of each SNP assayed and its position within each locus. Validation of the tetra-primer ARMS–PCR was possible because the allelic composition of Baronesse, Karl, Lewes, Morex, and Steptoe at each locus was known (Fig. 2). The primers were first

Table 1. Tetra-primer ARMS–PCR primers and conditions.

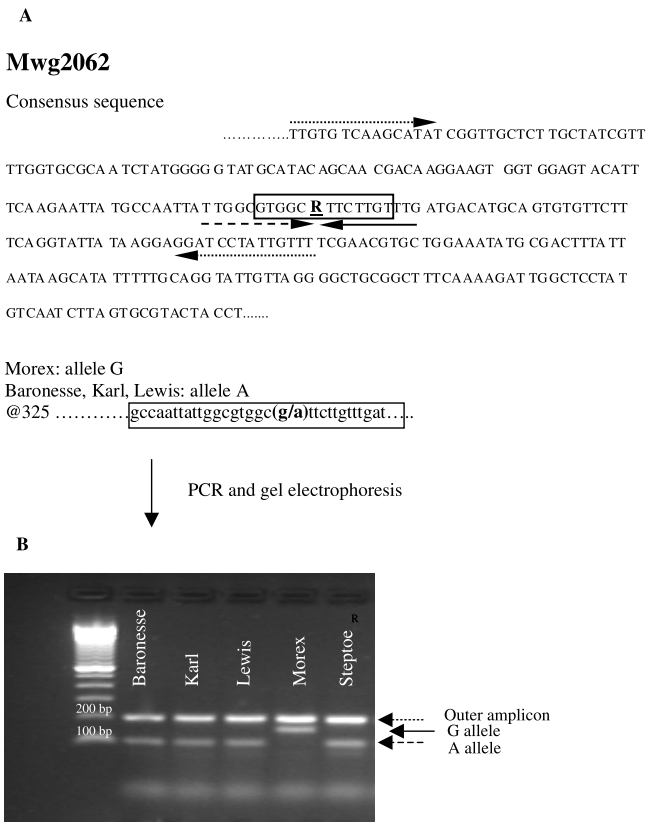
Genetic polymorphism		Primer description					
Locus – position – SNP	Primer sequences (5′→ 3′)	T_m (°C)	Secondary structure	Dimer	T_a (°C)[a]	T_a (°C)[b]	Amplicon size (bp)
MWG2062 – 325 – A–G							
Forward inner primer (A allele)	AAGAATTATGCCAATTATTGGCGTGTCA	70.5	Weak	No	72	65	A allele: 101
Reverse inner primer (G allele)	CACACTGCATGTCATCAAACAAGCAC	71.3	Weak	No	72	65	G allele: 151
Forward outer primer	GTTGTGTCAAGCATATCGGTTGCTCTT	70.5	None	No	72	65	
Reverse outer primer	CAGCACGTTCGAAAACAATAGGATCC	70.6	None	No	72	65	Two outer primers: 198
ABC465 – 254 – C–T							
Forward inner primer (T allele)	TGGAGATGTTCTACGCTCTCAAGTACAGT	68.6	Moderate	No	72	65	T allele: 130
Reverse inner primer (C allele)	CTGTTGGTCAGATAACCTACCAGGATG	68.1	Moderate	No	72	65	C allele: 162
Forward outer primer	CAGGTACACCTGGAAGCTCTACTCAGAG	68.8	Strong	No	72	65	
Reverse outer primer	CAGCAGCCTGAATTCAACAAAACATAC	68.9	Weak	No	72	65	Two outer primers: 236
MWG2218 – 175 – G–C							
Forward inner primer (C allele)	GGGGACGTCATCCACGTCTGTCGACC	79.3	Strong	No	72	65	C allele: 127
Reverse inner primer (G allele)	GTTCCCGCGGTGGGCTTTGTTTCCTC	79.3	Moderate	No	72	65	G allele: 140
Forward outer primer	CTCTCCGACATCGACCGCTTCCTCTTCG	79.1	Weak	No	72	65	
Reverse outer primer	GCCGCATCATCCCTGGTGTCATCACCT	79.8	Strong	No	72	65	Two outer primers: 215
MWG502 – 656 – A–G							
Forward inner primer (A allele)	GATGGCGTACCGAGGCGGCCAAAAAA	79.8	Strong	No			A allele: 237
Reverse inner primer (G allele)	GATTAGTTTGATGGATAATTAATCAGC	60.7	Weak	No			G allele: 265
Forward outer primer	GGGCTTTAATATCCGTGCTAACCGAATA	69.9	None	No			
Reverse outer primer	TAATTAATCGGCGTGAGAAGTTTCATGG	70.3	Very weak	No			Two outer primers: 449
ABG601 – 390 – C–T							
Forward inner primer (C allele)	CTTCTTAGTCTAAACTTCCATGTCGTTTAC	64.0	Weak	No	68	60	C allele: 230
Reverse inner primer (T allele)	TTAGGGCTACAACAAAATATCAAGGATA	64.1	Weak	No	68	60	T allele: 290
Forward outer primer	ACTCTGTTTACGCTCTTACTATAGGGCT	64.3	None	No	68	60	
Reverse outer primer	ACAGAGTACAACATTGGATTTAAGGAAG	64.1	None	No	68	60	Two outer primers: 462
ABG704 – 344 – A–G							
Forward inner primer (G allele)	GATGGACTGTCAGTAAATGACGTGGG	70.1	Moderate	No	72	65	G allele: 221
Reverse inner primer (A allele)	GTGAGGCAGGAAACCCACTAAGAAGAT	69.7	Weak	No	72	65	A allele: 196
Forward outer primer	AAAGCAAGAGTTTTGTGGGTCTTGGATA	69.7	Moderate	No	72	65	
Reverse outer primer	AAAACAAGCCTGAGCTTCCAGAGATTAG	68.9	Moderate	No	72	65	Two outer primers: 364
ABC156 – 231 – T–G							
Forward inner primer (G allele)	TCCATATAGGTCTCTCTTTTCTTATTATG	60.8	Weak	No			G allele: 70
Reverse inner primer (T allele)	TGAGAGACTCAATACTCATGAATTTCA	63.7	Moderate	No			T allele: 60
Forward outer primer	CTTGGTCCATATAGGTCTCTCTTTTC	63.0	None	No			
Reverse outer primer	CCTCCTGATATACTTGAGAGACTCAATA	62.6	Moderate	No			Two outer primers: 74
MWG2029 – 204 – A–G							
Forward inner primer (G allele)	GTTTTTTCTTCTTCTATATTGATGATTTTG	62.0	None	No	68	60	G allele: 225
Reverse inner primer (A allele)	CGACACCGGCACCTATATGCACCGGT	78.3	Very strong	No	68	60	A allele: 290
Forward outer primer	GAAACTTCTTTAGTTGAACGCGAATTGGA	70.5	Weak	No	68	60	Two outer primers: 459
Reverse outer primer	AGGAGAAGAGAGCAGTACCTCTCCCTGT	70.0	Strong	No	68	60	

Table 1 (*concluded*).

Genetic polymorphism		Primer description				
Locus – position – SNP	Primer sequences (5′→ 3′)	T_m (°C)	Secondary structure	Dimer	T_a (°C)[a]	T_a (°C)[b]	Amplicon size (bp)
MWG801 – 344 – G–A							
Forward inner primer (C allele)	GAAGCATGCTCGCACGACACCCATCC	79.8	Very weak	No	72	65	C allele: 175
Reverse inner primer (A allele)	CGGCAGCGGAGGGGAAGGGGAGCAGT	83.9	None	No	72	65	A allele: 133
Forward outer primer	CAACAACCCCAATACCAGGCCAGCTCCACA	80.9	None	No	72	65	Two outer primers: 256
Reverse outer primer	AACCCTCGACTGCTCAAGGCAGAGCCGC	81.5	Strong	No	72	65	

Note: T_m, melting temperature; T_a, annealing temperature.
[a]T_a of the first cycle.
[b]T_a of the remaining cycles.

Fig. 2. Validation of genotyping scores by tetra-primer ARMS–PCR. (A) Consensus sequence of locus MWG2062 showing the positions of identified SNPs in bold (R = A, G). The SNP assayed is bracketed below. Arrows indicate the position and sequences of the primers used. The allelic composition of the varieties ‘Baronesse’, ‘Karl’, ‘Lewis’, and ‘Morex’ is shown below the sequence. (B) Agarose gel electrophoresis for ‘Baronesse’, ‘Karl’, ‘Lewis’, ‘Morex’, and ‘Steptoe’.



tested on these varieties and the protocol optimized before genotyping was performed on the full barley variety set. Primer sets were considered validated when both allelic bands and the outer fragment, all of the predicted sizes, were visualized.

Results and discussion

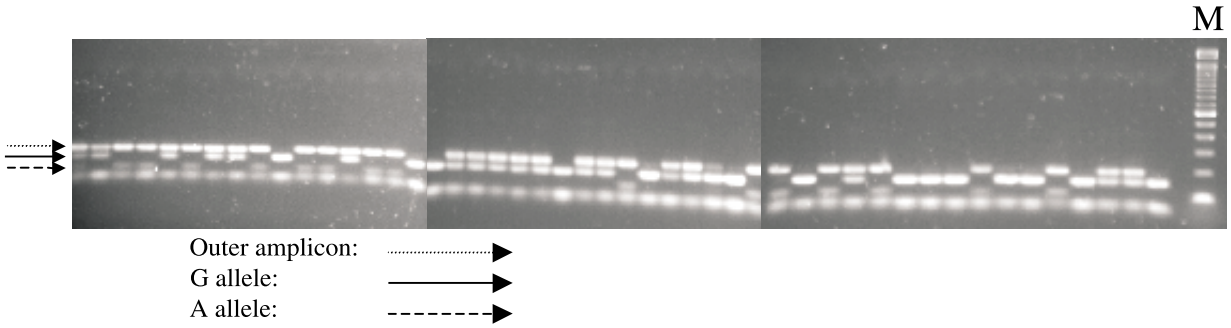
Tetra-primer ARMS–PCR is a rapid, simple, robust, low-cost, and easy to use method for SNP genotyping, which could be used even in “low-tech” laboratories. In this work, we have designed the primers to amplify fragments that differ in size sufficiently to be easily resolved by agarose gel electrophoresis. This modification from the original method makes the technique more readily usable across a range of laboratories and research capabilities.

We were able to genotype the five “test” varieties with only five out of nine primer combinations designed. Amplification experiments showed that the most important factor to be met to obtain the allele amplification is the T_m of the primers. Sets of primers with equal T_m but strong secondary structure gave better results than primers with weak or no secondary structure but different T_m. For instance, primers designed for genotyping the A–G SNP at locus MWG2029

Table 2. SNP genotyping summary for 132 barley varieties.

Locus	Chromosome	Polymorphism[a]	Varieties' allelic composition (ratio)
MWG2062	7H	R	80 G : 50 A
ABC465	7H	Y	123 T : 9 C
MWG2218	6H?	S	82 C : 50 G
MWG502	5H	R	78 A : 54 G
ABG601	4H	Y	89 C : 40 T

Fig. 3. Agarose gel electrophoresis of 48 barley varieties typed by tetra-primer ARMS–PCR at locus MWG2062. M, 100-bp marker.



amplified only the outer band for Steptoe, while both the outer and the expected inner bands were amplified for Karl (A allele). From the T_m values of these primers (Table 1), it could be inferred that during the first PCR cycles, the forward inner primer (G allele) was disadvantaged in the annealing, thus leading to the amplification of only the A-specific amplicon. The sequence available for locus ABC156 was only 310 bp in length, and no suitable primers could be found for genotyping the varieties using the parameters as outlined in the Materials and methods that were designed for scoring SNPs in agarose gels. Ye et al. (2001) demonstrated that altering the ratio of the outer to the inner primer (1:10) enhanced amplification of the allele-specific products and reduced artifacts. We noticed that this leads, in some cases, to outer fragment amplification failure. However, in these cases where only the inner amplicon was amplified, it was still possible to unambiguously genotype the samples (see Fig. 3).

The 132 varieties assayed represent a set of commercially successful varieties of winter and spring barley cultivated in the United Kingdom in the last 70 years. The genotyping results are given in Table 2 and an example of agarose gel electrophoresis of 48 barley varieties genotyped by tetra-primer ARMS–PCR is shown in Fig. 3. When the data are analysed, locus ABC465 shows a strong bias for one allelic form, as a ratio of 123 T : 9 C was found. A nucleotide BLAST search of this sequence reveals this locus to be a gene for sucrose synthase, an enzyme involved in starch synthesis (Amor et al. 1995). This nonrandom allele distribution may represent linkage to some character(s) that has undergone selection in the breeding programmes or an allelic bias in the founder genotypes entering such programmes. Analysis of the amino acid sequences around the SNP did not show any changes, suggesting that the mutation is silent. The allelic status of another locus (ABG 601) showed correlation with winter and spring barley types (Table 3). This could be explained by the linkage of ABG601 to a gene(s) determining the vernalization requirement of barley. This locus maps to chromosome 4H and is in the same BIN13 as the *Sgh1* locus (http://barleygenomics.wsu.edu/arnis/linkage_maps/ maps-svg.html) and it maps to the same BIN of the *Sgh1* locus, which is one of two loci (*Sgh1* and *Sgh2*) controlling the vernalization requirement (Laurie et al. 1995).

The utility of a neutral marker system depends, to a large extent, on its information content. Ching et al. (2002) inferred that individual SNPs are not very informative as molecular markers for use in genetic diversity studies because the heterozygosity value calculated on the basis of the haplotypes is lower for SNPs when compared with that from simple sequence repeats. However, the abundance of SNPs would more than compensate for this deficiency, in the presence of genotyping platforms that offer a compromise between high throughput and low costs. The results for locus ABG601 illustrate the potential for the use of SNPs in assessing varieties from the angle of performance or their value for cultivation and use (value for cultivation and use testing). For plant variety registration, the current distinctness, uniformity, and stability testing mainly relies on measurements of agronomic and phenotypic characteristics; the value for cultivation and use is also assessed by measuring variety performance for specific traits using non-DNA-based methods. The association of a specific SNP allele at locus ABG601 with the growth habit of barley varieties shows that it is possible to predict phenotypes without the need for growing the plants. However, before accepting a molecular marker as a predictive test for a phenotypic character, it is important to establish the haplotype structure at a given locus across a significant number of varieties covering a wide range of genetic material. Whole breeding and varieties testing procedures could be so complemented by a virtually infi-

Table 3. SNP alleles of the 132 barley varieties at locus ABG601.

Variety	Type	ABG601 allele
Alexis	S	C
Brewster	S	C
Camargue	S	C
Chad	S	C
Chariot	S	C
Cooper	S	C
Dandy	S	C
Derkado	S	C
Delibes	S	C
Felicie	S	C
Hart	S	C
HeronJIC	S	C
Nomad	S	C
Prisma	S	C
Triumph	S	C
Tyne	S	C
Atem	S	C
Corniche	S	C
Digger	S	C
Doublet	S	C
Joline	S	C
Klaxon	S	C
Natasha	S	C
Regatta	S	C
Apex	S	C
Delta	S	C
Egmont	S	C
Golf	S	C
Koru	S	C
Kym	S	C
Patty	S	C
Tasman	S	C
Abacus	S	C
Arimir	S	C
Ark_Royal	S	C
Armelle	S	C
Athos	S	C
Georgie	S	C
Goldmarker	S	C
Hassan	S	C
Jupiter	S	C
Keg	S	C
Lofa_Abed	S	C
Magnum	S	C
M_Mink	S	C
Mazurka	S	C
Midas	S	C
Porthos	S	C
Simon	S	C
Sundance	S	C
Tyra	S	C
Wing	S	C
Berac	S	C
Deba_Abed	S	C
Gerka	S	C
Imber	S	C
Proctor	S	C
Universe	S	C
Vada	S	C
Zephyr	S	C
Impala	S	C
Inis	S	C
M_Badger	S	C
Mosane	S	C
Sultan	S	C
Cambrinus	S	C
Freja	S	—[a]
M_Baldric	S	C
Pallas	S	T
Carlsberg	S	C
Standwell	S	—[a]
Golden_Pheasant	S	C
Forester	S	C
HeronNIAB	S	T
Spratt_Archer	S	C
Golden_Archer	S	C
Kenia	S	C
Maja	S	C
Union	S	C
Camton	S	C
Rika	S	C
Bronze	W	T
Epic	W	T
Fighter	W	T
Halcyon	W	T
Linnet	W	T
Marinka	W	T
Pastoral	W	T
Pipkin	W	T
Puffin	W	T
Sprite	W	T
Target	W	T
Willow	W	T
Gerbel	W	T
Igri	W	T
M_Otter	W	T
Pirate	W	T
Plaisant	W	T
Sonja	W	T
Tipper	W	T
Athene	W	T
Hopple	W	T
M_Trojan	W	T
Astrix	W	T
Mirra	W	T
Senta	W	T
M_Puma	W	T
Dea	W	T
Pioneer	W	T
Carlsberg2	W	C
Earl	W	C
Herta	W	C
Maythorpe	W	C

Table 3 (*continued*).

Table 3 (*concluded*).

Variety	Type	ABG601 allele
Provost	W	C
Plumage_Archer	W	C
Prefect	W	T
Bere	W	C
Six_Row_Winter	W	T
Victory	W	—[a]
Webbs_Sunrise	W	C
Clarine	W	T
Frolic	W	T
Gypsy	W	T
Kira	W	T
Libra	W	C
Magie	W	T
Melusine	W	T
Posaune	W	T
Torrent	W	T

Note: Varieties are divided into spring (S) and winter (W) types.
[a]Missing data.

nite set of molecular markers developed directly from the functional regions of the genome coding for agronomic and quality traits or from DNA sequences linked to such genes.

Acknowledgements

We thank Dr. T. Blake and V. Kanazin for supplying DNA samples, support, and assistance. This work was funded by the EC Framework Programme V, GEDIFLUX project (QLRT-2000-00934), and by the Marie Curie Host Fellowship scheme, contract No. QLK5-1999-50753.

References

Amor, Y., Haigler, C.H., Johnson, S., Wainscott, M., and Delmer, D.P. 1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc. Natl. Acad. Sci. U.S.A. **92**: 9353–9357.

Bhattramakki, D., and Rafalski, A. 2001. *In* Plant genotyping: the DNA fingerprinting of plants. *Edited by* R.J. Henry. CAB International Publishing, Wallingford, U.K. pp. 179–192.

Ching, A., Caldwell, K.S., Jung, M., Dolan, M., Smith, O.S., Tingey, S., Morgante, M., and Rafalski, A.J. 2002. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet. **3**: 19.

Donini, P., Stephensons, P., Bryan, G.J., and Koebner, R.M.D. 1998. The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genet. Resour. Crop Evol. **45**: 415–421.

Kanazin, V., Talbert, H., See, D., DeCamp, P., Nevo, E., and Blake, T. 2002. Discovery and assay of single-nucleotide polymorphisms in barley (*Hordeum vulgare*). Plant Mol. Biol. **48**: 529–537.

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**(2–3): 145–151.

Landegren, U., Nilsson, M., and Kwok, P.Y. 1998. Reading bits of genetic information: methods for single-nucleotide polymorphism analysis. Genome Res. **8**: 769–776.

Laurie, D.A., Pratchett, N., Bezant, J.H., and Snape, J.W. 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome, **38**: 575–558.

Rafalski, A. 2002. Applications of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol. **5**: 94–100.

Ridout, C.J., and Donini, P. 1999. Use of AFLP in cereals research. Trends Plant Sci. **4**: 76–79.

Ronaghi, M., Uhlen, M., and Nyren, P. 1998. A sequencing method based on real-time pyrophosphate. Science (Wash., D.C.), **281**: 363–365.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R.G., Mullis, K.B., and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science (Wash., D.C.), **339**: 487–491.

Schneider, K., Weisshaar, B., Borchardt, D.C., and Salamini, F. 2001. SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. Mol. Breed. **8**: 63–74.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **15**: 503–517.

Tenaillon, M.I., Sawkins, M.C., Long, A.D., Gaut, R.L., Doebley, J.F., and Gaut, B.S. 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). Proc. Natl. Acad. Sci. U.S.A. **98**: 9161–9166.

Useche, F.J., Gao, G., Harafey, M., and Rafalski, A. 2001. High-throughput identification, database storage and analysis of SNPs in EST sequences. Genome Inf. Ser. Workshop Genome Inf. **12**: 194–203.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

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.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535.

Ye, S., Dhillon, S., Ke, X., Collins, A.R., and Day, I.N. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res. **29**: e88.