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ORIGINAL PAPER

A genetic map of 1,000 SSR and DArT markers in a wide barley cross

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Abstract A high-density genetic map was developed from an F1-derived doubled haploid population generated from a cross between cultivated barley (*Hordeum vulgare*) and the subspecies *H. vulgare* ssp. *spontaneum*. The map comprises 1,000 loci, amplified using 536 SSR (558 loci) and 442 DArT markers. Of the SSRs, 149 markers (153 loci) were derived from barley ESTs, and 7 from wheat ESTs. A high level of polymorphism (~70%) was observed, which facilitated the mapping of 197 SSRs for which genetic assignments had not been previously reported. Comparison with a published composite map showed a high level of co-linearity and telomeric coverage on all seven chromosomes. This map provides access to previously unmapped SSRs, improved genome coverage due to the integration of DArT and EST-SSRs and overcomes locus order issues of composite maps constructed from the alignment of several genetic maps.

Keywords *Hordeum vulgare* spp. *spontaneum* · SSR · EST · DArT · Genetic mapping · Wild relatives

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Introduction

Molecular markers have played an important role in understanding the genetic basis of economically important traits in barley. Over the last decade, comprehensive genetic maps have been constructed for the seven barley chromosomes and have been used in QTL analysis (Kleinhofs et al. 1993), and to isolate genes through map based cloning (Kilian et al. 1997; Simons et al. 1997). An important use of markers has been marker assisted selection (MAS), which is made possible by the identification of markers linked to commercially important traits such as disease resistance (Graner et al. 1996), response or tolerance to abiotic stress (Forster et al. 2000) and seed or feed quality traits (Han et al. 1995). Initially, barley genetic maps were based on restriction fragment length polymorphisms (RFLPs), providing robust, co-dominant markers for all chromosome groups (Graner et al. 1991; Heun et al. 1991). However, RFLPs are technically complex, require large quantities of DNA and present a limitation to high throughput genetic analysis. More recently, trait linked RFLP markers have been adapted for PCR amplification, making them amenable to MAS (Tacconi et al. 2006). Barley genetic maps have also been constructed using simple sequence repeats (SSRs) (Ramsay et al. 2000; Thiel et al. 2003; Varshney et al. 2006), which are a preferred type of marker for cereal genetic analysis.

Simple sequence repeats, also known as microsatellites, are short tandem repeats of DNA (Lagercrantz et al. 1993). They are multiallelic, co-dominant and evenly distributed throughout the genome of eukaryotic species. Being PCR based, SSRs are technically simple to deploy and are amenable to high throughput assays (Mansfield et al. 1994). In recent years, an important use of SSRs has been MAS in early generation breeding populations (Gupta and Varshney

2000). Marker assisted breeding is generally more efficient when molecular maps are saturated, due to an increased chance of finding polymorphic markers in any genetic background. To date, approximately 1,000 barley SSRs have been published (Becker and Heun 1995; Liu et al. 1996; Pillen et al. 2000; Ramsay et al. 1999; Sagahi-Maroof et al. 1994; Struss and Pliescke 1998), of which about half have been genetically mapped (Ramsay et al. 2000; Varshney et al. 2006; Wenzl et al. 2006).

Detailed genetic maps are becoming increasingly important in theoretical and applied genetic research (Vuylsteke et al. 1999). The construction of genetic maps for barley with SSRs has been hampered by the limited polymorphism (~30%) that is typically found in crosses derived from cultivated germplasm (Ablett et al. 2003). This problem has been partially addressed by the generation of composite maps, constructed from multiple mapping populations (Ablett et al. 2003; Karakousis et al. 2003; Rostoks et al. 2005; Wenzl et al. 2006), to improve genome coverage and marker density. However, loci order can be difficult to establish when the number of common markers between individual maps is limited. This ambiguity is a limitation to certain types of genetic analyses such as linkage disequilibrium mapping and whole genome studies.

In the present study, a high density SSR and Diversity Array Technology (DArT) map was developed from a cross between cultivated barley (*H. vulgare*) and the wild relative *H. vulgare* ssp. *spontaneum*. This map serves to increase the pool of markers available to cereal breeders and researchers by genetically mapping previously uncharacterised SSRs, and provides a valuable resource for future studies in which knowledge of marker locus order is important.

Methods

Mapping population

An F1-derived doubled haploid (DH) population was created from a cross between the Australian feed variety barley, "Barque-73", and *H. vulgare* spp. *spontaneum* accession "CPI 71284-48" using isolated microspore culture. Barque-73 was reselected from the variety Barque to remove known heterogeneity. Both Barque-73 and CPI 71284-48 were selected for their differing osmotic adjustment capacities (Eglinton et al. 2000) to facilitate adaptation to low rainfall regions of Australia. The DH population consisted of 90 progeny. Fifteen lines were identified to be clonal in subsequent genetic analysis, reducing the population to 75 unique lines. The 15 clonal lines were used as controls for genetic mapping to assess the quality of marker data. Plants were grown in the green house and harvested for leaf tissue at the four to five-leaf-stage. Seed for the mapping population and parental lines is available for research purposes upon request.

Genetic analysis

DNA extractions were performed as described by Rogowsky et al. (1991) with minor modifications. Primer sequences for SSRs used in this study were obtained from the Graingenes database (http://www.wheat.pw.usda.gov/GG2/index.shtml), or by material transfer agreement. Details of publicly available primer sequences and allele size data are given in Supplementary Table S1. Contact information for proprietary sequences is also provided. Of the available SSRs for barley, 833 were assessed for polymorphism between the parents of the mapping population. Additionally, 68 wheat EST-SSRs amplifying orthologous fragments in barley were screened for polymorphism (P. Sourdille personal communication). The majority of SSRs were assayed using the Multiplex-Ready PCR Technology as described by Hayden et al. (2005) and analysed on an ABI 3730 using GeneMapper v3.7 genotyping software (Applied Biosystems). The optimal concentration of each primer pair amplified using Multiplex-Ready PCR is detailed in Supplementary Table S1. A small number of SSR markers were amplified using conventional PCR and resolved using poly acrylamide gel electrophoresis (PAGE) as described by Karakousis et al. (2003). Mapped loci amplified in this way are also indicated in the Supplementary Table S1. Diversity Array Technology® (DArT) marker assays were performed by Triticarte Pty Ltd (Australia) and used to provide additional genomic coverage.

Genetic mapping

A genetic map was constructed using MapManager QTXb20 (Manly et al. 2001). Initially, a framework map of high quality SSRs was constructed using the distribute function with $P = 0.0001$. The remaining markers were then manually placed by searching for linkages with the highest possible LOD score to minimise the number of apparent crossovers. The robustness of the locus ordering was verified using RECORD (Van Os et al. 2006). Final map distances were calculated using R/qtl (Broman et al. 2003) using the Kosambi mapping function, as MapManager QTXb20 was shown to underestimate the genetic distance between loci when missing genotypes were recorded. MapChart was used to illustrate the genetic map (Voorrips 2002).

The distribution of each marker type was determined by examining the frequency of markers within 10 cM intervals on each chromosome and testing for uniformity using a Chi-squared test. The genetic map was aligned with the Scottish Crop Research Institute (SCRI) composite map

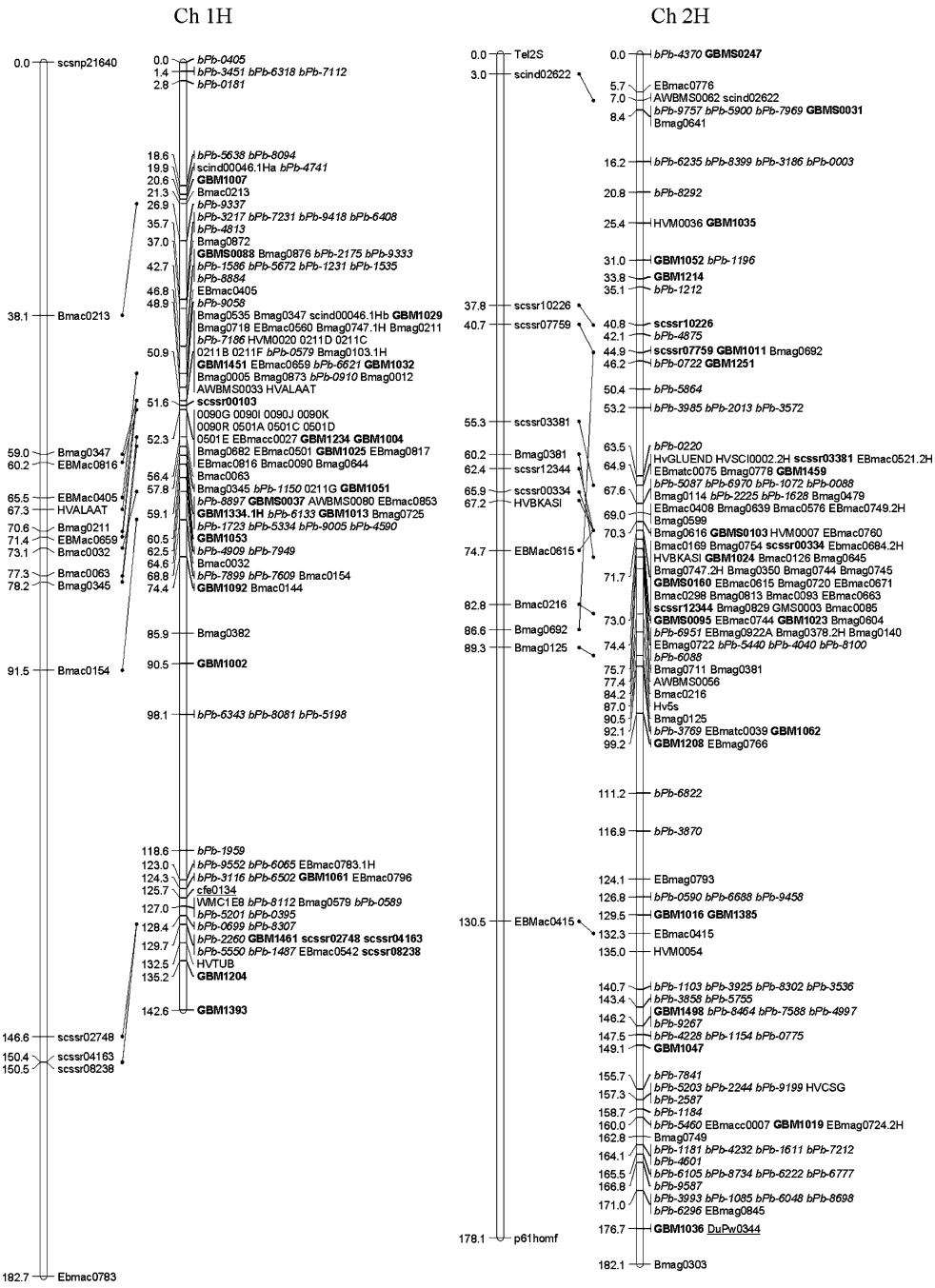


Fig. 1 Genetic linkage map of the Barque-73 × *H. vulgare* spp. *spontaneum* cross. Each chromosome is aligned with homologous loci on corresponding linkage groups of the SCRI composite map (Rostoks et al. 2005), which is shown on the left. DArTs begin with the prefix bPb and are shown in *italics*. EST-derived SSRs are shown in *boldface* type, and wheat EST-SSRs are *underlined*. Anonymous SSRs are in *plain text*. Chromosomes are orientated with the short arms at the *top*

(Rostoks et al. 2005) for comparison of genome coverage and locus order. For clarity, only homologous loci are displayed on the composite map. The mapping data has been provided in Supplementary Table S2.

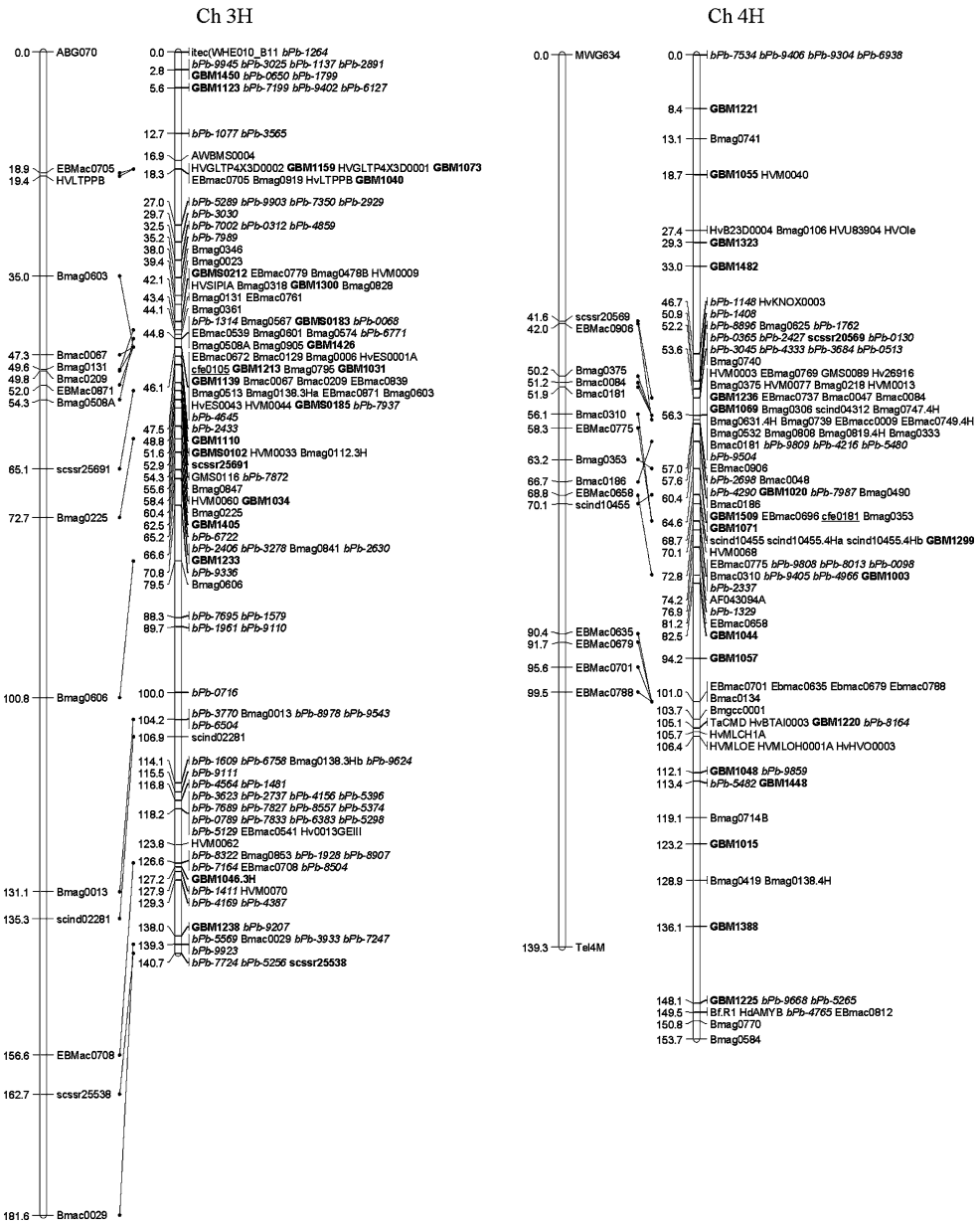
Marker nomenclature

Published SSRs are reported as a prefix followed by four digits (e.g. Bmac0001, Bmac1000) and multilocus SSRs are distinguished by the addition of the chromosome group after the marker name (e.g. Bmac0001.3H). Markers amplifying multiple loci on the same chromosome are denoted by a letter added to the end of the chromosome group (e.g. Bmac0001.3Ha).

Error checking

The 15 clonal DH lines were used to assess the repeatability of each marker assay. Erroneous assays identified from this process were visually inspected. The quality of the genetic map was assessed iteratively by looking for double crossovers using the functions described by Lehmensiek et al. (2005).

Fig. 1 continued



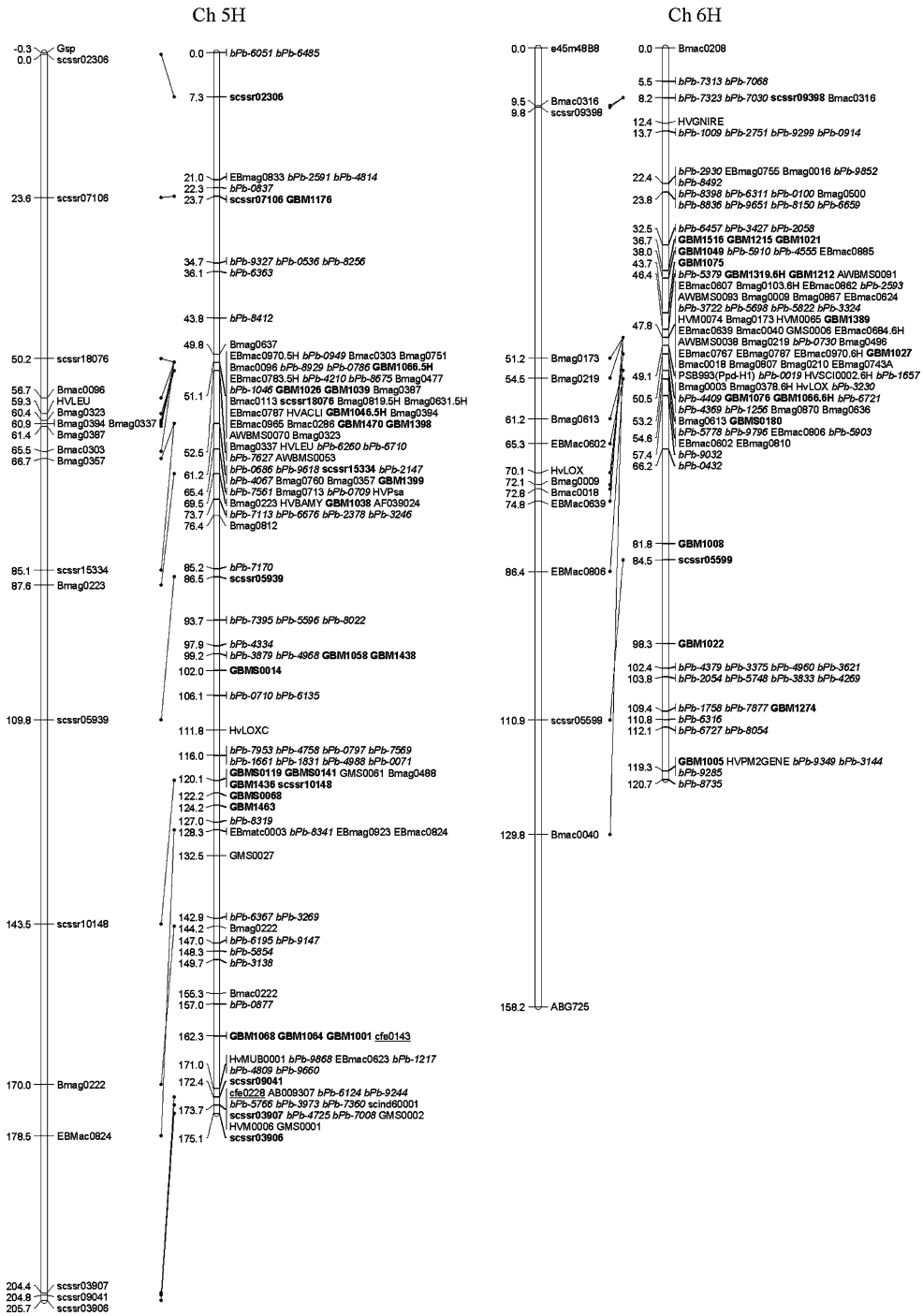
Marker map locations were also checked for discrepancies with published maps. Where chromosome group discrepancies were observed, the ABI electrotraces for the markers were re-examined and compared with a parental reference. In this way, errors resulting from markers with overlapping or similar allele sizes were resolved.

Results

Of the 1,002 available SSRs, 833 amplified PCR fragments that were suitable for genetic analysis on an ABI3730 DNA fragment analyser. Of these, 569 markers (~70%) revealed polymorphism between the parents of the mapping population. EST and anonymous SSRs revealed a similar level of polymorphism: 72 and 70%, respectively. In contrast, only 10% of the 68 wheat EST-SSRs exhibited polymorphism. Twenty barley SSRs amplified more than one polymorphic locus, two amplified more than two loci and three amplified two loci on the same chromosome. Of the markers amplifying multiple loci on the same chromosome, one amplified two co-segregating loci.

A total of 442 DArT and 558 SSR loci were integrated into the genetic map (Fig. 1). Of the SSR loci, 153 were amplified by 149 EST-SSRs, seven were amplified by seven wheat EST-SSRs, and 398 were amplified by 380 anonymous SSRs. The total length of the genetic map was 1,100.1 cM, with individual linkage groups ranging from 120.7 cM (6H) to 182.1 cM (2H). The largest intervals between markers were on the short and long arms of

Fig. 1 continued



chromosome 7H (21.3 and 41.0 cM, respectively). Alignment with the SCRI composite map (Fig. 1) showed a high level of conservation of SSR locus order with few exceptions (Rostoks et al. 2005). The composite map comprised 1237 SNP, AFLP, RFLP and SSR markers, although only homologous loci are illustrated. The alignment of the two maps showed that the Barque-73 × *H. vulgare* spp. *spontaneum* genetic linkage map provided a high level of genome coverage, comparable to a well saturated composite map.

Null alleles were revealed by 20 and 16% of anonymous and EST SSRs, respectively. The transferability of EST and anonymous SSRs to the *H. spontaneum* parent was 89% for both types of SSR marker. For the DArT markers, which are allele-specific, 70% transferability was observed. A high proportion of SSR and DArT data showed the

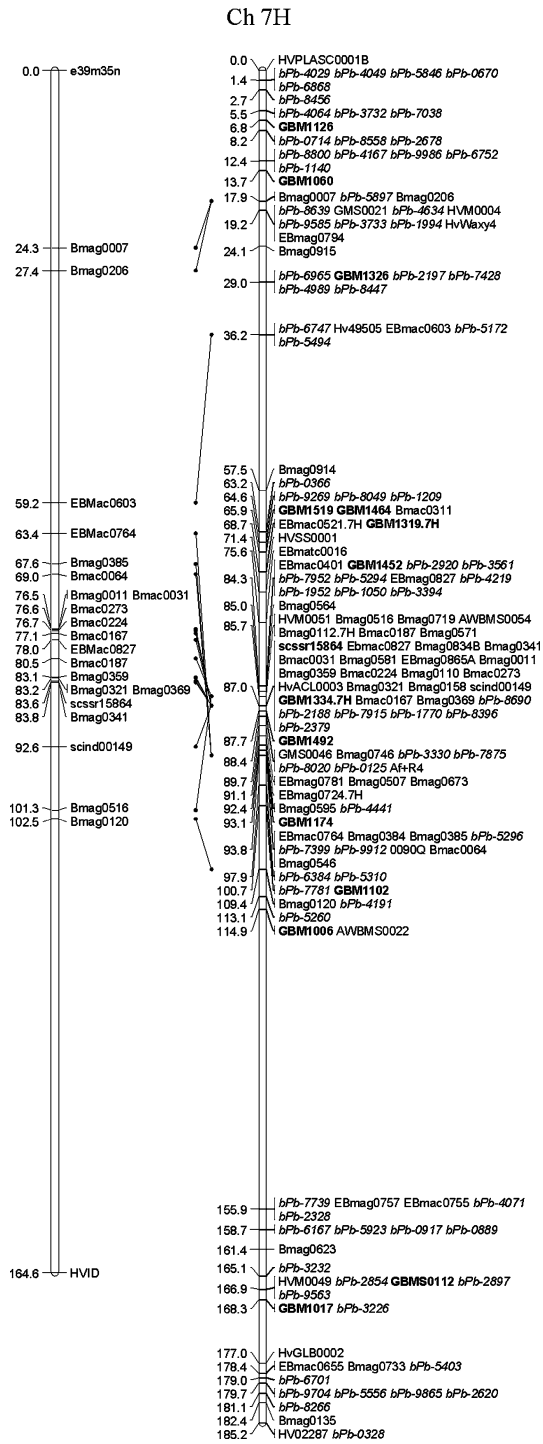


Fig. 1 continued

H. spontaneum allele to be under represented in the mapping population, with severe segregation distortion on 3HS ($P < 0.001$). Segregation distortion was observed to some extent on regions of all chromosomes.

Diversity Array Technology provided telomeric coverage on four chromosome arms (1HS, 4HS, 5HS and 6HL) that extended beyond mapped SSR loci. Chi squared tests revealed a significant difference between the chromosomal distribution of DArT and SSR markers, and between EST and anonymous SSRs. The DArTs were significantly less clustered than SSRs, with EST-SSRs being significantly less clustered than anonymous SSRs ($P < 0.0001$) (Fig. 2). Each chromosome contained at least one bin (i.e. a group of markers not separated by recombination) of more than 20 markers at the approximate location of the centromere. Six chromosomes contained at least one bin with more than 30 markers (Fig. 1).

Discussion

The genetic map described provides a valuable resource for barley genetic analysis. Containing 558 SSR and 442 DArT loci, the map provides sufficient genome coverage for whole genome analysis and a greater choice of markers to tag chromosomal regions of interest (Fig. 1). The use of a wide barley cross allowed a large proportion of SSRs (~70%) to be mapped in a single population. Crosses between more closely related germplasm typically reveal a lower level of polymorphism. For example, 12 Australian barley mapping populations were required to achieve 78% polymorphism, which is comparable to that achieved in the present study (Ablett et al. 2003). Improved SSR density on the current map was also facilitated by fluorescent-based marker detection and capillary electrophoresis (Hayden et al. 2005). This assay platform provides higher resolution for small allele size differences and multilocus markers compared to non-denaturing PAGE, which has been typically used in previous mapping studies (Karakousis et al. 2003).

The majority (529) of SSRs reported in this study amplified a single, polymorphic fragment for a number of reasons. First, use of the MultiplexRready PCR technology allowed the PCR specificity of individual markers to be optimised for the amplification of target loci by adjusting the concentration of locus specific primer for each marker (Hayden et al. 2005). Second, the inclusion of EST-SSRs (153) may have contributed to the low number of multilocus markers observed due to the fact that they are derived from highly conserved, transcribed regions (Chabane et al. 2005). Indeed, EST-SSRs are reported to behave principally as single-locus markers in mapping populations (Varshney et al. 2006). In the present study, only four EST-SSRs were mapped to more than one location. In previous studies, these EST-SSRs were mapped to only single locations (Varshney et al. 2006), which is likely due to limited polymorphism in the mapping populations used (Thiel et al. 2003). The observation that the majority of SSRs mapped to a single locus in the present study is in agreement with the published locations for most of these markers in other mapping populations.

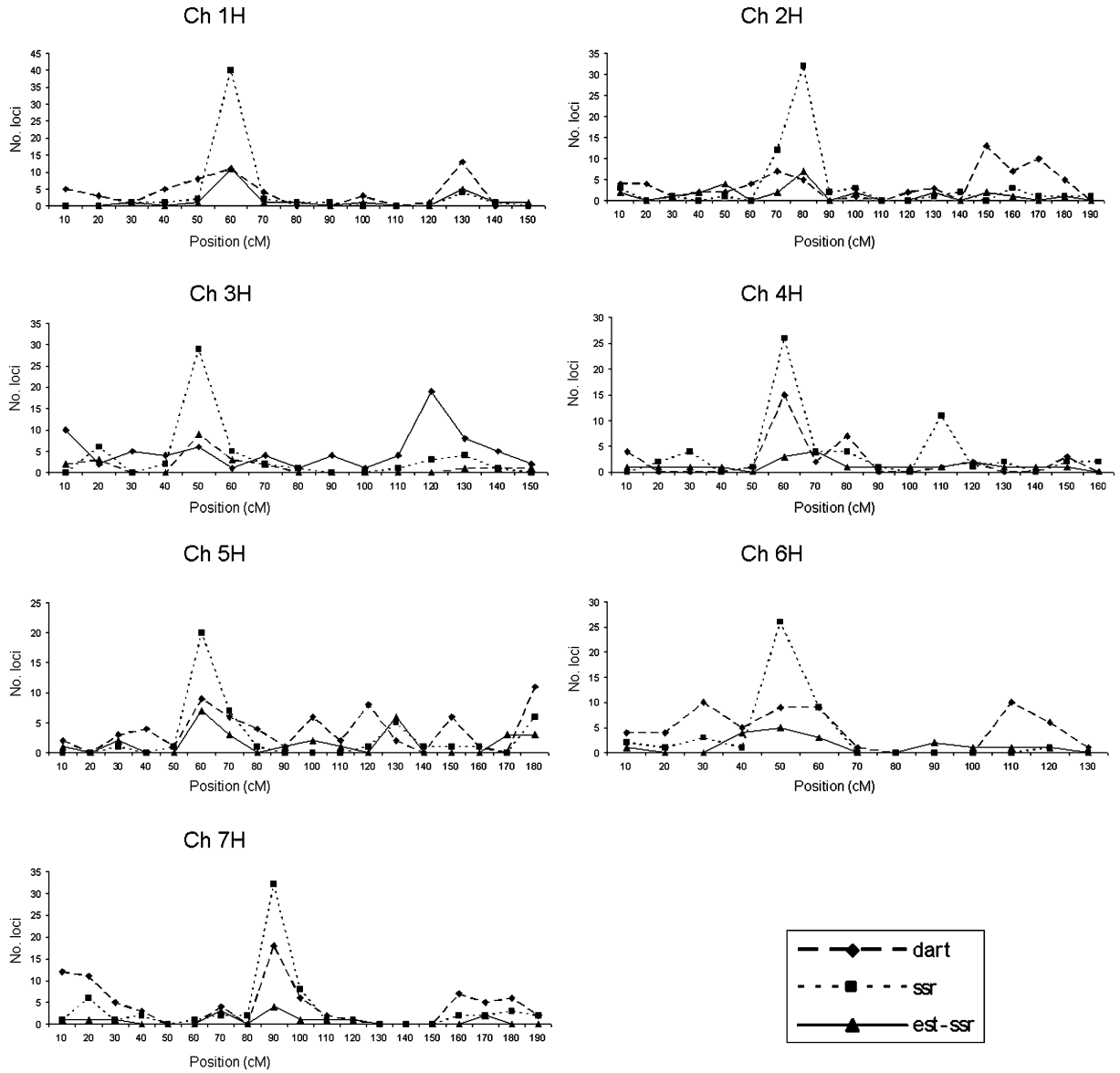


Fig. 2 Distribution of DArT, EST-SSR and anonymous SSR loci along each chromosome group of the Barque-73 × *H. vulgare* spp. *spontaneum* genetic map

Comparison of the genetic map with a published composite map showed a high level of genome coverage, including the telomeric regions (Fig. 1). This resulted largely from the inclusion of DArT and EST-SSRs. These markers typically show improved genome coverage compared to anonymous (non-coding) SSRs, which are characteristically clustered around the centromeric regions (Ramsay et al. 2000). This difference in genome coverage is thought to reflect the processes used to develop each type of marker. Anonymous SSRs are usually developed from random genomic libraries, in which microsatellites located in the heterochromatic regions are over-represented (Roder et al. 1998). In contrast, an iterative process of selection and rearraying of polymorprhic DArT clones is used to specifically enhance genome coverage (Wenzl et al. 2004), and the development of EST-SSRs from genic regions reduces the representation of regions that are rich in repetitive DNA (Parida et al. 2006). Despite the improved distribution achieved in the current map, some clustering was observed at the centromeric region of each chromosome. Nevertheless, the genome coverage achieved makes the present map particularly useful to select markers for use in whole genome breeding strategies and saturate genomic regions of interest. A high level of transferability of anonymous and EST derived SSRs (89% each) to the *H. spontaneum* parent indicates that both types of markers will be useful in genetic studies involving *H. spontaneum* germplasm (Supplementary Table S1). In contrast, DArT

markers showed a lower level of transferability between the sub species, with only of 30% of the DArT alleles detected being inherited from the *H. spontaneum* parent. The low level of *H. spontaneum* alleles detected in this study is consistent with DArT technology providing species-specific markers (Wenzl et al. 2004).

The relatively small size of the mapping population ($n = 75$) limited the likelihood of observing recombination between closely linked marker loci. As a consequence, closely linked (<5 cM) markers that may have been genetically separated on other published maps could not be resolved in the present population and are represented as bins of co-segregating markers (Fig. 1). As expected, the largest bins of co-segregating markers were observed at the centromeres, a region known to have suppressed recombination (Kunzel et al. 2000). However, resolution of closely linked markers is not always necessary and depends on the type of genetic analysis being undertaken. For many types of genetic studies, these unresolved regions will provide access to a high density of markers and thereby increase the chance of finding markers that are polymorphic in specific germplasm. This will be particularly useful in plant breeding to facilitate the transfer of QTL between different genetic backgrounds. In studies where marker loci order is important the combined use of the present map with published composite maps will be more useful. Composite maps provide greater resolution for marker locus order by virtue of genetic recombination that is achieved across multiple mapping populations (Marcel et al. 2007). In the present map, co-segregating loci provide a high level of certainty that marker scores are correct, since the markers are essentially replicates of each other. As such, there is certainty in the relative order of maker bins along a chromosome. This is an advantage over composite maps where the order of markers may differ between individual component maps, making it difficult to determine the true loci order. Moreover, composite maps often do not represent all of the markers mapped in the individual populations (Karakousis et al. 2003). An advantage of the current map is that markers contained within regions of limited resolution can be used to more accurately infer the location of markers that were unable to be integrated into a composite map.

In conclusion, the present map provides a valuable resource for barley genetic research and contains the largest collection of anonymous and EST-derived SSRs mapped in a single barley population, including many SSRs (~200) whose chromosomal location was previously unknown. In particular, this map has expanded the pool of markers available for barley research, and will facilitate future studies including marker-assisted breeding, association mapping, whole genome selection, comparative mapping and map based cloning.

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