A high-density genetic map for barley integrating SSR and DArT markers

PR. Hearnden[1,2], PJ. Eckermann[1,2], MJ. Hayden[1,2] , GL. McMichael[1,2], JK. Eglinton[2], and KJ. Chalmers[1,2]

The University of Adelaide, Waite Campus, Glen Osmond, SA, 5064, Australia.
[1] Molecular Plant Breeding Cooperative Research Centre (MPBCRC)
[2] School of Agriculture and Wine, The University of Adelaide

Abstract:

A high density genetic map comprising 246 SSR and 184 unique DArT loci was constructed using an F1-derived DH population developed from a cross between cultivated barley (*H. vulgare*, variety Barque) and the closely related species, *H. spontaneum* (accession CPI 71284-48). The parents of this cross revealed a high (68 per cent) level of polymorphism which facilitated the mapping of previously unmapped markers and an unprecedented level of genetic saturation.

Comparisons with published maps showed a high level of co-linearity, although some discrepancies in loci order exist between closely linked markers. This map provides access to previously unmapped SSRs, improved genome coverage due to the integration of DArT and SSR markers and overcomes loci order issues of consensus maps constructed from the alignment of several genetic maps. In addition, the use of *H. spontaneum* in the cross will aid the introgression of novel traits into adapted barley germplasm.

Key Words: *Hordeum vulgare*; *Hordeum spontaneum*; SSR; DArT; genetic mapping; wide cross.

Introduction:

Microsatellite markers, or simple sequence repeats (SSRs) have been shown to rapidly and efficiently identify barley genotypes, even among closely related cultivars (12). Approximately 1000 microsatellite markers have been developed for barley (13, 2, 6, 12, 8, 10), with approximately 300 represented in published linkage maps. Their relative abundance, high level of polymorphism and low cost of assays have made SSRs the preferred marker for barley genetic research. SSRs have been used to construct genetic maps for several Australian barley populations (1) and have been integrated into linkage maps comprising AFLP and RFLP markers (5). Consensus maps produced from multiple populations has allowed the potential to identify SSR markers in genomic regions of interest, which were previously identified by the more labour intensive RFLP and AFLP markers.

Despite the abundance and polymophic nature of SSRs, current linkage maps comprise relatively few of the available SSR markers for barley, leaving a large, untapped resource of informative PCR based markers. Telomeric regions have been poorly represented with clustering occurring around the centromere (10). Inadequate coverage of the barley genome will hamper marker assisted selection and map based cloning of genes governing important quality traits.

A new genetic map is presented in this paper, developed from a cross between the subspecies *H.vulgare* ssp. *spontaneum* and the related species *H. spontaneum*. The use of a wide cross produced high levels of polymorphism, allowing previously unmapped SSR markers to be placed on the map. A combination of SSR and diversity array technology (DArT) markers has been used to produce a highly saturated genetic map of barley. In addition to the increased level of polymorphism, the use of *H. spontaneum* will enable introgression of desirable QTL from this wild relative into cultivated barley.

Methods:

Plant material
An F1-derived Doubled Haploid (DH) population was derived from a cross between an Australian feed variety barley, “Barque-73”, and the *Hordeum spontaneum* accession “CPI 71284-48”. Both Barque-73 and CPI 71284-48 were selected for their differing osmotic adjustment capacities (3), to facilitate adaptation to the low rainfall regions of Australia. Barque-73 was employed as the female parent in the cross, with isolated microspore culture used to develop the DH plants from F1 plants. A population of 90 DH plants was

produced. Fifteen of the lines were assessed in duplicate leaving a population of 75 unique lines. Plants were grown in trays in the green house and harvested for leaf tissue at the four to five-leaf-stage.

Genotyping
DNA extractions were performed as described by Rogowsky et al. (11) with minor modifications. Four hundred SSR markers were assayed using MultiplexReady technology (4). Over 400 DArT markers were subsequently assayed to provide further saturation of the genome. The DArT assays were conducted by Triticarte Pty Ltd.

Genetic mapping
A genetic map for the DH cross was constructed using the package MapManagerQTXb20 (7). Initially, a framework map was constructed using SSR markers and a LOD value of 5. The distribute function was then used to assign the remaining SSR markers to linkage groups. Where necessary the linkage criterion was lowered to join linkage groups belonging to the same chromosome. DArT markers were assigned to chromosome using the distribute function and marker orders determined using the ripple command. Several markers were manually placed as needed. The quality of the genetic map was assessed iteratively by looking for double crossovers and manually checking marker scores where necessary and reassessing marker loci orders. Map distances were imported into JoinMap for construction of chromosome illustrations. Multilocus SSRs were distinguished by the addition of the chromosome group name after the marker name.

Results:

Genotyping
Of the SSR markers assayed, 242 produced robust and polymorphic products. One hundred and forty nine of the primers assayed produced ambiguous profiles or produced products that were too faint to score. One hundred and fifteen SSR markers were monomorphic, producing a polymorphism rate of 68 per cent. Three SSR primers amplified more than one loci. Approximately five per cent of the SSR primers amplified a null allele in one of the parents. The majority of null alleles came from the *H. sponaneum* parent.

Genetic mapping
Seven linkage groups corresponding to each of the barley chromosomes were constructed from the 242 polymorphic SSRs. After integration of the DArT markers, a total map length of 1063cM was achieved (Figure 1). Severe segregation distortion was observed along the short arm of chromosome 3H where ratios of up to 1:6 ($p<0.00000001$) were observed in favour of the Barque-73 allele.

Two hundred and forty six loci, amplified by the 242 polymorhpic SSRs, and 435 DArT markers were successfully integrated into the F1-derived DH map. Forty seven and 58 per cent for the SSR and DArT markers cosegregated with one or more markers respectively. For the purpose of map illustration, redundant DArT markers were omitted. Comparison with published marker data revealed a high level of co-linearity, with the exception of closely linked markers.

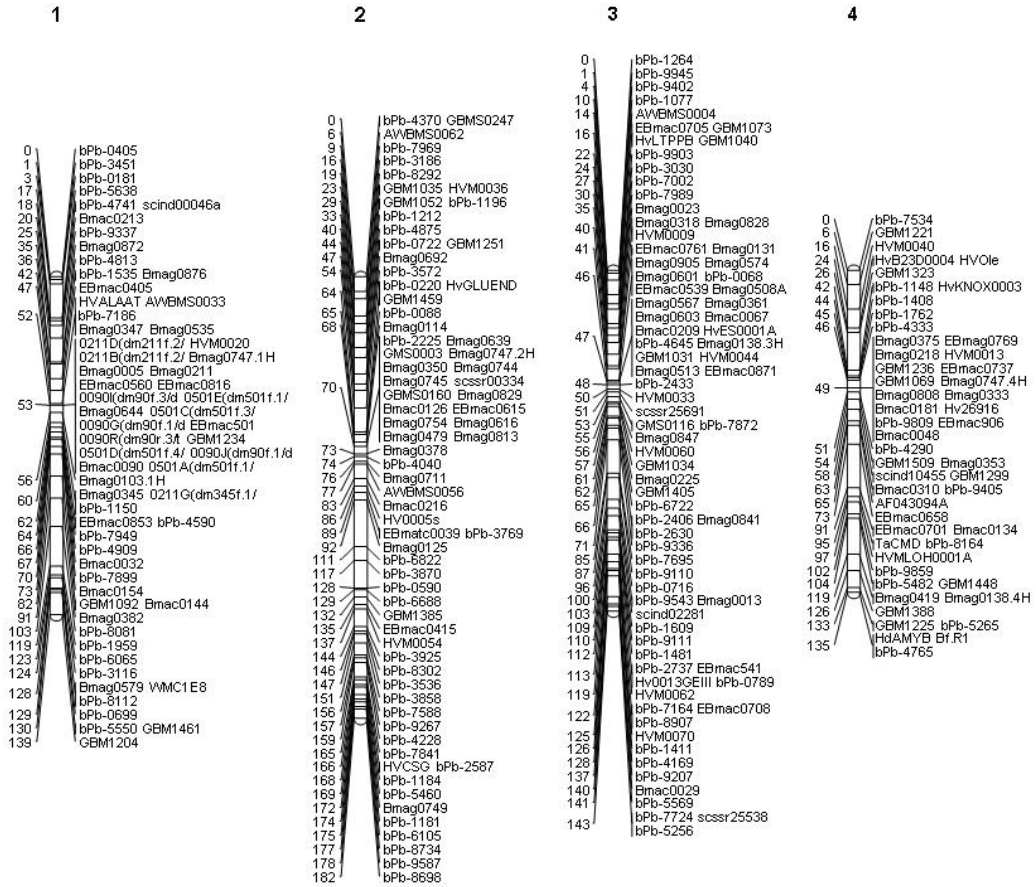

Figure 1: Genetic linkage map of an F1-derived DH population derived from the cross Barque-73/*H. sponaneum.* Short arms of chromosomes are at the top. Multilocus SSR markers are distinguished by their chromosome group after the marker name. The distances shown on the left of each chromosome are in centimorgans (Kosambi).

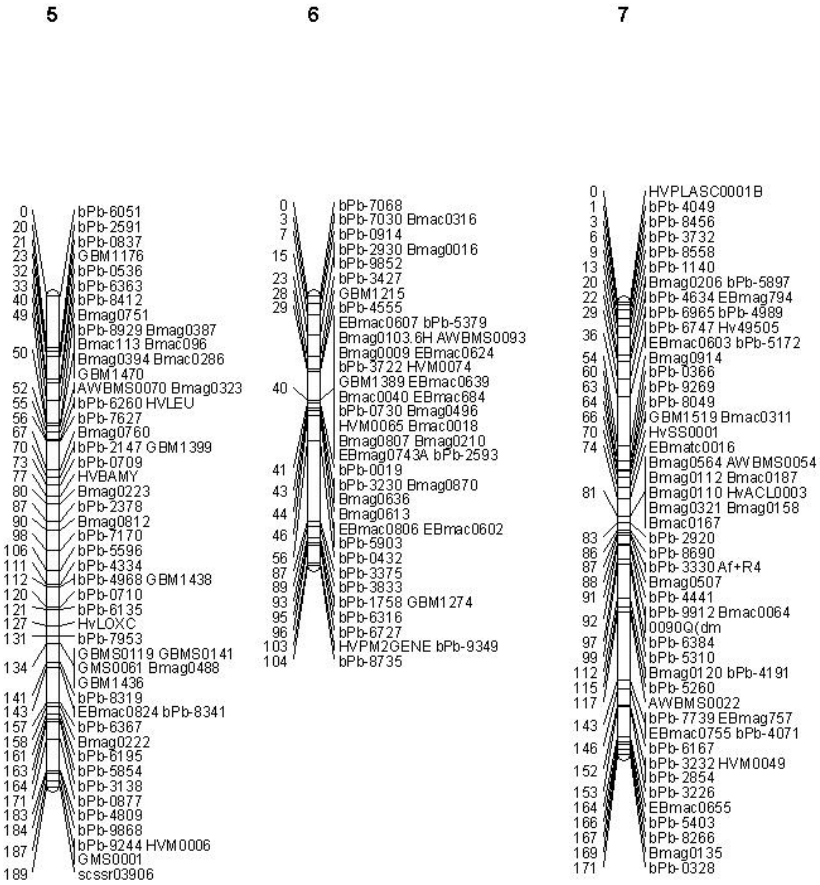

Figure 1: -Continued.

Discussion:

The genetic map derived from the Barque-73/ *H. spontaneum* cross presented here comprises 246 SSR markers and 184 unique DArT markers. A further 251 DArT markers have been mapped but were not included here due difficulty in displaying multiple redundant markers. As such, the positions of 681 loci are currently available. The total map length of the Barque-73/ *H. spontaneum* cross was 1063cM (Kosambi). This is slightly less than the distance reported by Ramsay et al., (10) for a DH population derived from an interspecific barley cross (1173cM Kosambi). Although several SSR markers were shown to co-segregate on this map, they most likely represent unique loci which were not separated by recombination in this population.

The relatively small population size (75) of the DH population limits the likelihood of observing recombination between closely linked loci (Table 1). Consequently, the loci order for tightly linked loci (<5cM) may not be correct. However, the accurate placement of markers within a 5cM distance will enhance map based cloning efforts and marker assisted selection. Assessment of 15 of the DH lines in duplicate confirmed the accuracy of marker genotyping and the success of the error checking process used to construct the genetic map. The high level of co-segregation observed may be due to the random selection of SSR markers. SSR markers were selected primarily on the basis of allele size and quality, for efficient capillary packing. Multilocus markers were under represented as they were more difficult to resolve in conjunction with other markers. Marker selection is often, and understandably, based on genome coverage. This may explain the relatively low level of multilocus markers observed (three out of 242) compared with the study by Ramsay et al., (10) who reported 27 SSRs out of 242 amplifying more than one product. As genotyping of SSRs continues in this population, more multilocus markers will be included.

Table 1: Chance of observing no recombination between loci in a segregating population of 75 individuals.

Genetic Distance Between Markers (cM)	Chance of no recombination events between markers
1	47.06%
2	21.98%
5	2.13%
10	0.04%
20	<0.00%

Telomeric regions were under represented by the SSRs in this study. Although some clustering was observed, the addition of DArT markers provided more even coverage of the telomeric regions. Centromeric clustering has been previously observed in mapping studies involving SSRs in barley (10). In contrast, DArT markers have been shown to offer greater telomeric coverage in an intraspecific cross (14). Centromeric clustering of markers has been considered the result of the distribution of recombination events in interspecific crosses (10). Non random distribution of SSRs has also been suggested as a possible cause (9), and is the likely explanation for the distribution of the different marker types in the linkage map presented here. The majority of SSRs were derived from non-coding sequences. Such SSRs are known to be unevenly distributed due an association with retroelements (9).

The majority of SSR and DArT data showed the *H. spontaneum* allele to be under represented in the DH population. In addition, several null alleles were identified in the SSR data, most of which were conferred by the *H. spontaneum* parent. Severe segregation distortion on 3HS ($p<0.001$) may indicate the existence of tissue culture suppression loci conferred by the *H. spontaneum* parent. The existence of segregation distortion is considered common among DH populations. Segregation distortion has been reported in a similar DH cross around the centromere of 2H (10). Segregation distortion was observed to some extent on all chromosomes in the present study, including the centromereic region of 2H ($p<0.05$).

Improved resolution of existing SSRs with Multplex Ready technology (4) has enabled single base pair polymorphisms to be resolved, thus increasing the available number of polymorphic SSRs. The ability to adequately score poor quality and multilocus markers has hampered saturation levels in previous mapping studies (5). The level of polymorphism observed in the *H. vulgare* x *H. spontaneum* cross was 68 per cent, which exceeds the level of polymorphism observed in most conventional barley mapping populations. Twelve barley populations were required to achieve comparable polymorphism levels (78 per cent) in a previous study (1).

Conclusion:

The Barque-73/*H. spontaneum* linkage map is the first of its kind to integrate SSR and DArT markers and comprises more loci than is currently available for published barley linkage maps. Despite the relatively small population size employed in this cross, the ability to accurately place markers in “bins” of closely linked markers (~5cM) will aid the efforts of those wishing to clone genes linked to traits of interest. The high polymorphism rate of the Barque-73/*H. spontaneum* population, combined with improvements in SSR resolution and throughput, will allow placement of the majority of available SSR markers for barley on a single map.

Acknowledgments:

The authors gratefully acknowledge the financial support of the Grains Research Development Corporation and the Molecular Plant Breeding CRC. The authors also acknowledge the University of Adelaide for hosting the research and the members of the molecular marker laboratory for their ongoing support.

References:

(1) Ablett, G.A., Karakousis, A., Banbury, L., Cakir, M., Holton, T.A., Langridge, P., & Henry, R.J. (2003). Application of SSR markers in the construction of Australian barley genetic maps. Australian Journal of Agricultural Research. 54: 1187-1195.

(2) Becker, J., & Heun, M. (1995). Barley microsatellites-allele variation and mapping. Plant Molecular Biology. 27: 835-845.

(3) Eglinton, JK., Jefferies, SP., Ceccarelli, S., Grando, S., McDonald, G. and Barr AR. (2000). A comparative assessment of osmotic adjustment and oxidative stress responses in Hordeum vulgare ssp. spontaneum. Proceedings of the 8th International Barley Genetics Symposium, Adelaide, Australia 3: pp.267-269.

(4) Hayden M.J., Nguyen T. & Chalmers. K.J. (2005) Multiplex-Ready Markers. A technique for high-thoughput, low-cost genotyping on an automated DNA fragment analyser. Proceedings of the 12th Annual Barley Technical Symposium.

(5) Karakousis, A., Gustafson, J.P., Chalmers, K.J., Barr, A.R., & Langridge, P. (2003). A consensus map of barley iintegrating SSR, RFLP, and AFLP markers. Australian Journal of Agricultural Research. 54: 1173-1185.

(6) Liu, Z.W., Biyashev, R.M., & Maroof, M.A.S. (1996). Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics. 93: 869-876.

(7) Manly, K.F., Cudmore, Jr. R.H., & Meer, J.M. (2001). Map Manager QTX, cross-platform software for genetic mapping. Mammalian Genome 12: 930-932.

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

(9) Ramsay, L., Macaulay, M., Cardle, L., Morgante, M., degli Ivanissevich S., Maestri, E., Powell, W., & Waugh, R. (1999). Intimate association of microsatellite repeats with retrotransposons and other dispersed repetitive elements in barley. Plant Journal. 17: 415-425.

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

(11) Rogowsky, P.M., Guidet, F.L.Y., Langridge, P., Sheperd, K.W. and Koebner, R.M.D. (1991). Theor Appl Genet 82:527-544.

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

(13)Sagahi-Maroof, M.A.S., Biyashev, R.M., Yang, G.P., Zhang, Q., & Allard R.W. (1994). Extraordinarily polymorphic microsatellite. DNA in barley-species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences of the United States of America. 91: 5466-5470.

(14)Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinjofs, A., and Killian, A. (2004). Diversity arrays technology (DArT) for whole-genome profiling of barley. Proceeding of the National Academy of Sciences of the United States of America. 101: 9915-9920.