GENETIC MAPPING OF TRAITS IMPORTANT IN BARLEY BREEDING

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Contents

Abstract

Molecular markers were used for assessing genetic diversity in Finnish six-rowed barley and for mapping and tagging genes affecting traits important in barley breeding. Finnish six-rowed barley germplasm is narrow-based: twenty two released varieties are largely composed of only seven ancestors. The level of diversity in the RAPD markers has remained during barley breeding. Coancestry based on pedigree information and Jaccard's index based on RAPD markers were not correlated. A doubled haploid progeny from a cross between two Finnish six-rowed barley varieties (Rolfi and Botnia) was used for linkage map construction and genetic mapping. The map covered only 654 cM, probably due to genetic similarity of the parental varieties. Segregation distortion was detected in several chromosomal regions. When a set of doubled haploid lines were tested for their anther culture response, associations between anther culture traits and markers were detected. Only some of the associated markers were located on the chromosomal regions with distorted segregation. Anther culture traits have not been mapped previously in barley.

The Rolfi x Botnia linkage map was used for mapping agronomically important quantitative traits. From one to seven quantitative trait loci (QTLs) affecting each trait were detected. Many of these QTLs overlapped with QTLs found previously in other germplasm. Candidate loci were identified for QTLs affecting earliness and straw length. Clustering of QTLs was clear. Since many QTL clusters were situated in centromeric areas of the chromosomes, clustering may be explained by suppression of recombination. QTL x environment interactions and epistatic interactions were noted.

To facilitate introgression of resistance genes into Finnish barley germplasm, genes for net blotch resistance were mapped and a gene for powdery mildew resistance was tagged with DNA-markers. Two genes controlling net blotch resistance in the Rolfi x CI9819 cross were found: one with a major effect on chromosome 6H and a second with a smaller epistatic effect on chromosome 5H.

Information about QTLs underlying the genetic variation in agronomic traits can be utilized in barley breeding for mating design and selection. However, before using the putative QTLs in breeding, the exact locations and effects should be verified. Linked markers for net blotch and powdery mildew resistance may be used to speed up transfer of resistance from unadapted sources to the highly adapted elite Finnish barley germplasm.

Abbreviations

AP-PCR	arbitrarily primed PCR
BAC	bacterial artificial chromosome
BaMMV	barley mild mosaic virus
BaYMV	barley yellow mosaic virus
BC_1	first backcross generation
BSA	bulked segregant analysis
BYDV	barley yellow dwarf virus
C	carbon
CAPS	cleaved amplified polymorphic sequence
CCN	cereal cyst nematode
cDNA	complementary DNA
CIM	compound interval mapping
cM	centiMorgan
cpDNA	chloroplast DNA
CTAB	cetyltrimethyl-ammoniumbromid
DAF	DNA amplification fingerprinting
DH	doubled haploid
DNA	deoxyribonucleic acid
E	environment
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
F_1	first generation after a cross
F_2	second generation after a cross
F_3	third generation after a cross
Hja	Hankkija
IRAP	inter-retrotransposon amplified polymorphism
ISSR	inter-simple sequence repeat amplified polymorphism
ITEC	International Triticeae EST Co-operative
Jo	Jokioinen
LOD	logarithm of odds
MAAP	multiple arbitrary amplicon profiling
MAS	marker assisted selection
mRNA	messenger RNA
N	nitrogen
NIL	near isogenic line
P	phosphorus
PCR	polymerase chain reaction
QTL	quantitative trait locus
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SCAR	sequence characterized amplified region
sCIM	simplified composite interval mapping
SIM	simple interval mapping
SNP	single/simple nucleotide polymorphism
SSR	simple sequence repeat
STS	sequence tagged site
USDA	United States Department of Agriculture
YAC	yeast artificial chromosome

List of original publications

This thesis is based on the following original articles, which will be referred to in the text with Roman numerals.

I Manninen O. & Nissilä E. 1997. Genetic diversity among Finnish six-rowed barley cultivars based on pedigree information and DNA markers. Hereditas 126: 87-93.

II Manninen O. M. 2000. Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics 100: 57-62.

III Manninen O. M., Turpeinen T. & Nissilä E. 1997. Identification of RAPD markers closely linked to the *mlo*-locus in barley. Plant Breeding 116: 461-464.

IV Manninen O., Kalendar R., Robinson J. & Schulman A. 1999. Application of *BARE*-1 retrotransposon markers to map a major resistance gene for net blotch in barley. Manuscript, submitted.

V Manninen O. M. & Nissilä E. 1999. Mapping QTL and QTL x environment interactions for pre-heading and post-heading duration and agronomic traits in an elite spring barley cross. Manuscript, submitted.

1. Introduction

1.1 Barley

1.1.1 Taxonomy and origin

Cultivated barley, *Hordeum vulgare* L., belongs to the tribe *Triticeae* in the grass family, *Poaceae*. *Poaceae* is the largest family of monocotyledonous plants. The *Hordeum* L. genus comprises 32 species and altogether 45 taxa (Bothmer et al. 1991). It has been suggested that *H. vulgare*, together with *H. bulbosum* L., should be separated into a genus of its own, but this view has not been widely accepted (Bothmer 1992). The progenitor of barley is considered to be a subspecies of cultivated barley: *H. vulgare* subs. *spontaneum* (C. Koch) Tell. Both cultivated and wild barley have winter and summer annual forms. Barley can be divided into two-rowed and six-rowed types according to spike morphology; intermediate types also exist. In two-rowed barley the lateral spikelets are female sterile, while in six-rowed barley all spikelets are fertile (Briggs 1978).

The most widely accepted hypothesis on the origin of cultivated barley defines the Fertile Crescent as its centre of origin (Harlan 1976), but a hypothesis of multicentric origin has also been proposed (Molina-Cano et al. 1999). Data from cpDNA analysis suggests that barley has been taken into cultivation more than once, but that only very few domestication events have occurred (Zohary 1999, Neale et al. 1988).

1.1.2 Cultivation and use of the barley crop

Barley is a short season, early maturing grain with a high yield potential, and may be found on the fringes of agriculture, in widely varying environments (Harlan 1976). In Finland spring barley is cultivated at the northern species margin. Harsh winter conditions hinder cultivation of winter barley (Mukula & Rantanen 1989). Barley is among the five most important crop plants of the world. In 1997 barley was cultivated on 66 million hectares and yielded 157 000 million kg of grain. In Finland barley is the most important crop plant: 550 000 hectares were sown to barley in 1998, being 29% of the total area of crops, and the total yield was 1300 million kg (Yearbook of farm statistics 1998).

Barley grain is used to make malt, which in turn is used to make beer, whisky and some other products. In Western countries most barley grain is used to feed farm animals – cattle, sheep, goats, pigs, horses and poultry. In Eastern countries large quantities of barley are used in human food and drink (Briggs 1978). In Finland most of the barley grain is used for feeding pigs and cows but a marked portion is used for brewing malts (Grain

Bulletin 1999). In Finland barley is used for human consumption mainly as beer, bread and porridge.

1.1.3 The barley genome

Barley is a self-pollinating diploid with 2n = 2x = 14 (Bothmer 1992). The genome of barley has been estimated to contain around 5.5 picograms of DNA per haploid nucleus, equivalent to approximately 5.3×10^9 bp (Bennett & Smith 1976). In barley, as in other cereals, the genome consists of a complex mixture of unique and repeated nucleotide sequences (Flavell 1980). Approximately 10-20 % of the barley genome is tandemly arranged repeated sequences while 50-60 % is repeated sequences interspersed among one another or among unique nucleotide sequences (Rimpau et al. 1980). The interspersed *copia*-like retrotransposon *BARE*-1 comprises almost 7 % of the barley genome (Manninen & Schulman 1993).

Current estimates of gene number in higher plants vary between 25 000 and 43 000 (Miklos & Rubin 1996). In barley, a gene density of one gene per 123-212 kb can be expected if genes are distributed equidistantly (Panstruga et al. 1998). However, grass genomes seem to contain regions that are highly enriched in genes with very little or no repetitive DNA (Feuillet & Keller 1999, Barakat et al. 1997). Panstruga et al. (1998) found three genes on a 60 kb strech of DNA around the powdery mildew resistance locus, *mlo*, and Feuillet and Keller (1999) five genes on a 23 kb DNA around the receptor-like kinase gene, *Lrk10*.

1.1.4 Barley breeding

Breeding new barley varieties is based on creating new allele combinations and subsequent testing and selection of the desirable phenotypes during the selfing generations. Heritable variation is created mainly by controlled crosses between adapted high yielding cultivars and breeding lines. Although variety breeding is based on elite germplasm, specific traits may be introgressed from wild barley and landraces in backcrossing programs (Nevo 1992). Spontaneous mutations, as well as mutations induced by radiation or chemical treatments, have also been used (Briggs 1978). Recently, transgenosis has been added to the tools for creating new variation in barley (Ritala et al. 1994, Wan & Lemaux 1994).

Selection for desirable traits is made both in the field and in the laboratory. In the field agronomical characters including earliness, straw length, lodging resistance and disease resistance are monitored. After harvest yield, thousand grain weight, hectolitre weight and grading are measured as well as the protein content of the grain. Also malting properties including extract yield, viscosity of grain and malt, milling energy and diastatic power may be tested. Selection for specific traits is done during the selfing generations starting from

the F_2 generation. In a breeding program several traits have to be considered simultaneously to reach the desired agronomical type.

The early generations following crossing are highly heterozygous, making reliable selection difficult until an acceptable level of homozygosity is reached. A short cut to homozygosity can be achieved in barley by producing doubled haploid lines either from the immature pollen grains by anther or microspore culture, or through interspecific crosses between barley and *H. bulbosum* with subsequent chromosome elimination (Pickering & Devaux 1992). Both methods are used in commercial barley breeding programs and several doubled haploid varieties have been released.

1.2 **DNA-markers in barley breeding**

1.2.1 DNA-markers

Several different types of DNA markers are currently available for genetic analysis and new marker types are being developed continuously. Markers differ from each other in many respects: the initial workload and costs for building up the marker system, running costs and ease of use, level of polymorphisms, dominance, number of loci analyzed per assay, reproducibility and distribution on the chromosomes. Detection of polymorphism at the DNA level is usually based either on restriction patterns or differential amplification of DNA. The choice of the best marker system depends on whether it will be used in evolutionary or population studies, genetic mapping or fingerprinting. The ploidy level and reproductive system of the organism studied are also important. A comparison of DNA-markers used in barley is shown in Table 1.

Restriction fragment length polymorphism (RFLP) was first used for creating a linkage map in humans by Botstein et al. (1980) and the first applications in plant breeding were proposed by Burr et al. (1983). RFLPs are visualized after Southern blotting (Southern 1975) by hybridization to labelled DNA probes and subsequent autoradiography. Differences in the restriction patterns are caused by single nucleotide mutations at the restriction site or by longer deletions/insertions between restriction sites. A genomic or cDNA library is needed as a source of single or low copy probes. Probes from closely related species are applicable: for barley, clones from wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.) and rice (*Oryza sativa* L.) may be readily used. RFLP probes are useful as anchor markers for comparative studies within or between species and have been used for comparative mapping in the grass genera (Van Deynze et al. 1998, Devos & Gale 1997). Cloned genes with a function related to the trait of interest, and thus representing candidate genes, may be used as probes in mapping (Causse et al. 1995, Faris et al. 1999).

Table 1. Comparison of different DNA-marker systems. Modified from Rafalski & Tingey (1993), Kalendar et al. (1999) and Ridout & Donini (1999).

	RFLP	RAPD	SSR	AFLP	REMAP
Principle	Restriction Southern blotting Hybridization	DNA amplification with random primers	PCR of simple sequence repeats	Restriction Ligation of adapters Selective PCR	PCR of DNA between retro-transposons and SSR
Type of polymorphism	Single base changes Insertions Deletions	Single base changes Insertions Deletions	Changes in number of repeats	Single base changes Insertions Deletions	Single base changes Insertions Deletions
Level of polymorphism	High	Medium	Very high	Medium	High
Dominance	Codominant	Dominant	Codominant	Dominant	Dominant
Number of loci analyzed per assay	1-2	5-10	1	100-150	20-35
DNA required per assay	2-10 µg	20 ng	50 ng	0.5-1.0 µg	20 ng
Sequence information required?	No	No	Yes	No	Yes
Development costs	High	Low	High	Medium	Medium
Running costs per assay	Medium	Low	Medium	Medium	Low
Repeatability	Very high	Fair	Very high	Very high	High
Ease of use	Labour intensive	Easy	Easy	Difficult initially	Easy

Markers based on differential amplification of DNA can be divided in two groups based on the primer sequences used in a polymerase chain reaction, PCR (Mullis & Faloona 1987, Saiki et al. 1985). Methods using arbitrary primers have been collectively named Multiple Arbitrary Amplicon Profiling, MAAP (Caetano-Anollés et al. 1992). These include Random Amplified Polymorphic DNA, RAPD (Williams et al. 1991), Arbitrarily Primed Polymerase Chain Reaction, AP-PCR (Welsh & McClelland 1990) and DNA Amplification Fingerprinting, DAF (Caetano-Anollés et al. 1991). Depending on the method, arbitrary primers of 5-32 nucleotides are used for amplification, and different methods of separation and visualization of the fragments are used. The template DNA or the amplified fragments can further be cleaved by restriction enzymes to reveal additional

polymorphism (Riede et al. 1994). AFLP technique (Vos et al. 1995) combines MAAP with RFLP analysis in a special way: restriction fragments are ligated to adaptors and a selective PCR amplification of these fragments with hemispecific primers in PCR is performed. The complex amplification patterns are resolved on sequencing gels. All MAAP methods including AFLP produce dominant markers: heterozygotes cannot be distinguished from homozygotes expressing a band.

The other type of PCR based markers uses specific primers for amplification of DNA. These markers have generally been named Sequence Tagged Sites (STS, Olson et al. 1989, Inoue 1994). Sequence information has to be available to design primers for these applications. Specific primers have been produced in plants to analyze microsatellites or Simple Sequence Repeats, SSRs (Wu & Tanksley 1993), RFLP probes (Tragoonrung et al. 1992), RAPD fragments (Paran & Michelmore 1993), AFLP fragments (Shan et al. 1999) or expressed sequence tags (Bouchez & Höfte 1998).

Polymorphic microsatellites were first utilized in studies of humans (Tautz 1989) and later in plant studies (Morgante & Olivieri 1993). The SSR markers are based on amplification of a microsatellite using primers corresponding to specific flanking sequences and the length of the amplified fragments differs according to the number of di-, tri- or tetranucleotide repeats in the microsatellite sequence. A high number of alleles is typical for SSR markers, which makes them especially suitable for population studies (Goldstein & Pollock 1997). Up to 37 alleles have been reported in the HVM4 microsatellite locus of barley (Saghai Maroof et al. 1994). Sequence information for SSR amplification is obtained either from gene bank data or by sequencing positive clones probed from DNA libraries with simple sequence repeats. Currently, specific primer sequences for over 600 barley SSR loci are available (R. Waugh, personal comm.). Techniques based on random amplification of microsatellite sequences have also been proposed (Gupta et al. 1994, Wu et al. 1994). For barley a specific approach utilizing the *BARE*-1 retrotransposon LTR sequence as well as SSR sequences has been developed (Provan et al. 1999, Kalendar et al. 1999). Recently, primers based on the conserved regions of sequenced resistance genes have been used for amplifying resistance gene analogs (RGA) in many crop species, including barley (Leister et al. 1996, Chen et al. 1998).

Plant breeding programs require a genetic diagnostic assay that is relatively inexpensive and can be performed on thousands of individuals. All steps in the genetic diagnostic assay including DNA extraction, DNA quantification, amplification reaction, allele analyses and data read out, should be automated for fast output (Rafalski & Tingey 1993). Several methods are availabe for quick and easy purification of plant DNA for PCR (Langridge et al. 1991, Saini et al. 1999, Wang et al. 1993, Thomson & Henry 1995). PCR based methods are amenable to automatization of the genome analysis with pipetting robots as well as with computerized image analysis (Hodgson 1994). Recently developed DNA microarrays or DNA chips will allow simultaneous analysis of thousands of polymorphisms in a single experiment. Microarrays can be used for expression analysis,

polymorphism detection, DNA resequencing and genotyping on a genomic scale (Lemieux et al. 1998). Recently, DNA arrays based on single/simple nucleotide polymorphisms (SNPs) were used for rapid genome wide mapping in *Arabidopsis thaliana* L. (Cho et al. 1999).

1.2.2 Fingerprinting and diversity studies

The ability to discriminate between and identify varieties of agricultural crops is central to the operation of seed trade. Plant breeders rights offer protection for varieties, but in turn require that new varieties are distinct from others, uniform and stable in their characteristics (the so called D, U and S criteria) (Cooke 1995). Varietal identification and purity are also important for consumers, and especially for industry which uses the harvested yield for large-scale processing such as malting. Varietal identification in barley has long been based on morphological traits of the seed, seedling and the mature plants, supplemented with isoenzyme and hordein tests. Lately, DNA markers have been introduced as a promising method of fingerprinting barley varieties. For example, DNA fingerprints of all 65 registered six-rowed barley varieties in Canada have been generated using RAPD markers. All varieties could be identified from each other based on 18 polymorphic bands (Baum et al. 1998). AFLP and RAPD markers have also been successfully used for barley malt fingerprinting (Faccioli et al. 1999).

DNA polymorphisms can also be used to explore issues of genetic diversity. Knowledge of genetic diversity and the genetic relationship between genotypes is an important consideration for efficient rationalization and utilization of germplasm resources. Molecular markers can be used for constructing core collections of unrelated germplasm instead of a random collection (Hintum van 1994) and screening for duplicate accessions in germplasm collections (Virk et al. 1995). Information on genetic diversity is also needed for the optimal design of plant breeding programmes, influencing the choice of genotypes to cross for the development of new populations.

Molecular approaches have been used to group barley cultivars into morphologically distinct groups and further into subgroups that have a similar genetic background. RFLPs (Melchinger et al. 1994, Graner et al. 1994, Casas et al. 1998), RAPDs (Dweikat et al. 1993, Tinker et al. 1993), AFLPs (Hayes et al. 1997, Schut 1997, Ellis et al. 1997) and SSRs (Dávila et al. 1999, Russell et al. 1997) have all been used for assessing variation in local and global collections of barley germplasm. The mechanism of mutation generating new alleles in each marker system differs from each other and consequently affects the patterns of variability revealed (Powell et al. 1996). Differences may also reflect the fact that marker classes explore, at least in part, different portions of the genome (Noli et al. 1997).

1.2.3 Linkage maps

Construction of a genetic linkage map is based on observed recombination between marker loci in the experimental cross. Segregating families, e.g. F_2 or BC_1 progenies, F_3 families or single seed descent lines are commonly used. In barley the use of doubled haploid progenies produced from the F_1 generation simplifies genetic analysis. Doubled haploid lines have undergone only one meiotic cycle and carry a completely homozygous chromosome set. This means that the genetic information per plant is constant irrespective of the marker system used (Graner 1996).

Genetic map distances are based on recombination fractions between loci. The Haldane or Kosambi mapping functions are commonly used for converting the recombination fractions to map units or centiMorgans (cM). The Haldane mapping function takes into account the occurrence of multiple crossovers but the Kosambi mapping function accounts also for interference, which is the phenomenon of one crossing-over inhibiting the formation of another in its neighborhood (Ott 1985). Computer programs performing full multipoint linkage analysis include Mapmaker/Exp (Lander et al. 1987) and JoinMap (Stam 1993).

The early linkage maps of barley were based on morphological markers, later isozyme markers were added to the maps (reviewed by von Wettstein-Knowles 1992). The first DNA marker maps of the barley genome were published in 1991 (Heun et al. 1991, Graner et al. 1991). These maps, as well as the Steptoe/Morex map (Kleinhofs et al. 1993), were predominantly based on RFLP markers. Later, several barley maps based on other kinds of markers have been developed. These include linkage maps based on RAPD markers (Giese et al. 1994), SSRs (Liu et al. 1996), AFLPs (Qi et al. 1998, Becker et al. 1995), STSs (Mano et al. 1999) and randomly amplified SSRs (Dávila et al. 1999). Many other segregating progenies have also been used to construct partial maps and to determine locations of interesting genes. Markers associated with telomeres have been identified for most of the chromosomes (Kilian et al. 1999). In addition, integrated barley maps, based on segregation information of several independent doubled haploid progenies have been produced (Qi et al. 1996, Sherman et al. 1995). These consensus maps are useful when locations of genes are compared in crosses lacking common markers. The total genetic length of the barley maps ranges from 970 cM to 1873 cM, the length of the most comprehensive consensus map being 1060 cM. In the consensus map the lengths of the seven linkage groups range from 131 to 195 cM. One cM on the barley maps corresponds to approximately 1000-5000 bp. However, the genetic distances in the barley genome are not directly translatable to physical distances. Recombination appears less frequent in the centromeric regions of the chromosome arms (Pedersen & Linde-Laursen 1995) implying that a 1 cM distance in the distal part of the arm corresponds to a shorter physical distance than 1 cM in the proximal part of the arm. The marker order in the different barley maps is highly conserved and major differences in the genetic lengths of the homologous intervals are rare (Graner 1996). Comparative mapping within the *Poaceae* family has also revealed high levels of conservation of gene order (Devos & Gale 1997).

1.2.4 Tagging or mapping qualitative traits

Qualitative genes are inherited in a Mendelian fashion and their allelic forms give qualitatively distinct phenotypes. The phenotypes in a segregating progeny can be scored in a similar fashion as molecular markers. A normal segregation analysis will reveal linkages to any of the markers. Mapping a gene to a certain location on the chromosomes demands a linkage map of the whole genome, but genes can also be tagged with molecular markers without any previous information of the map location of markers used. Two approaches have been proposed for this purpose: use of near-isogenic lines, NILs (Martin et al. 1991, Muehlbauer et al. 1988), and of pooled DNA samples (Michelmore et al. 1991).

NILs differ only by the presence or absence of the target gene and a small region of flanking DNA. Hundreds of arbitrarily primed PCR-based markers can easily be screened to identify differences between isogenic lines and these differences are likely to be linked to the target gene. In barley the NILs have been used to tag a powdery mildew resistance gene (Hinze et al. 1991) and a spot blotch resistance gene (Hakim 1996).

In bulked segregant analysis (BSA) DNA pools of individuals of a crossing progeny are made based on their phenotype and screened for differences in the molecular markers (Michelmore et al. 1991). As a result of linkage disequilibrium, segregating markers that are tightly linked to the locus affecting the phenotype will most likely be fixed within the pool, while weaker linkage will result in both marker alleles being present. In barley BSA has successfully been used for tagging several disease resistance genes with RAPD markers locating 1.6-12 cM from the target locus (Weyen et al. 1996, Borovkova et al. 1997, Poulsen et al. 1995, Barua et al. 1993). BSA has also been proposed for tagging quantitative loci with a major effect: theoretically QTL alleles with phenotypic effects of 0.75-1.0 standard deviations should be detectable in DH populations of 100-200 lines (Wang & Paterson 1994).

1.2.5 Mapping quantitative trait loci

1.2.5.1 Quantitative traits

Characters exhibiting continuous variation are termed quantitative traits. Continuous variation is caused by two factors: simultaneous segregation of many genes affecting the trait and/or environment influencing the expression of the trait (Falconer & Mackay 1996). In crop plants most traits of economical importance, including yield, earliness, height and many quality traits, are quantitative. The unknown loci of the genes affecting these traits are commonly referred to as quantitative trait loci (QTL). Biometrical approaches have traditionally been used for studying quantitative traits and the statistical quantitative genetic model assuming essentially infinitely many genes with tiny effects works well for

many applied purposes, such as plant breeding. The details of the genetic basis of quantitative traits however remained unclear until the generation of complete genetic maps based on DNA markers.

1.2.5.2 Methods of QTL mapping

Association of morphological markers with quantitative traits in plants was observed early on (Sax 1923, Everson & Schaller 1955) and the first steps towards mapping of QTLs or polygenes were taken based on the scarce markers available (Thoday 1961). Currently, complete genetical maps exist for many crop species and algorithms have been developed for QTL mapping in a wide range of pedigrees and experimental designs including F_2, backcross, recombinant inbred, doubled haploid and many other designs (Paterson 1995). All share the basic principle of testing association between marker genotypes and quantitative phenotypes.

The most simple methods were based on single marker analysis, where the difference between the phenotypic means of the marker classes are compared using F-statistics, t-tests, linear regression or nonparametric tests (Sax 1923, Edwards et al. 1987, Soller & Brody 1976). A major shortcoming of single marker analysis is that it cannot distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect (Lander & Botstein 1989). The use of flanking markers for mapping made location of QTLs possible in the intervals between markers as well as at the marker sites. In interval mapping based on maximum likelihood methods (Lander & Botstein 1989) or multiple regression (Haley & Knott 1992) the test statistics for the presence of a putative QTL can be plotted along the chromosomes to present the evidence for QTLs at the various positions of the genome. The computer program Mapmaker (Lander et al. 1987) has been used extensively for performing interval mapping in plant studies. Interval mapping, now called simple interval mapping (SIM), searches for a single target QTL throughout a mapped genome. When multiple QTLs segregate, the sampling error associated with detection of a QTL may be inflated by the effects of other QTLs and furthermore, linked QTLs can cause biased estimates of QTL position (Tinker & Mather 1995a). Several methods fitting multiple QTLs, and based on nearly identical genetical concepts, have been proposed (Jansen 1993, Zeng 1994, Rodolphe & Lefort 1993). With these composite interval mapping (CIM) methods, the genetic variance caused by QTL other than the target is absorbed by the partial regression coefficients of the background markers. Several software packages are available for performing CIM: MapQTL and QTL Cartographer use maximum likelihood methods while MQTL, PLABQTL and MapManager are based on multiple regression. A comprehensive list of software for linkage and QTL analysis can be found at http://www.stat.wisc.edu/biosci/ linkage.html#linkage.

The significance thresholds used for reclaiming a QTL are of major importance. Because QTL mapping involves many analyses of independent genetic markers throughout the genome, there are many opportunities for false-positive results. The appropriate threshold

for controlling the type I error rate depends on the size of the genome and on the density of markers genotyped: a LOD threshold of 2.4 was considered adequate in SIM for a genome of 1100 cM covered with markers every 20 cM (Lander & Botstein 1989). This threshold was deduced from an assumed distribution for the test statistics, but the true distribution may deviate from the assumed distribution due to random distribution of the markers on the map (Tinker & Mather 1995a). Alternate methods are based on resampling: permutation involves shuffling the phenotypes so that the effects of the parameters are lost and the distribution of test statistics under the null hypothesis can be derived from repeated permutations (Churchill & Doerge 1994).

The power of finding a QTL can be increased by decreasing the variation caused by the environment as well as by the background genome. Environmental variation can be decreased by repeated phenotype measurements or by using progeny testing for phenotype measures (Lander & Botstein 1989). The power of QTL detection also depends on the type and numbers of progeny studied. Based on computer simulation studies, progeny sizes from a few hundreds to a thousand have been suggested to detect QTLs of minor effect. In practical barley studies, doubled haploid progenies of 100-200 lines have frequently been used for mapping purposes. The density of the marker map is not as important as the progeny size: a map with 50 cM marker spacings is adequate for detection of QTLs (Darvasi & Soller 1994). A more dense map helps to locate the QTLs more precisely (Darvasi et al. 1993).

Recent advances in QTL mapping procedures include analysis of QTL x environment interaction (Tinker & Mather 1995a,b, Jansen et al. 1995, Korol et al. 1998), a nonparametric approach to map QTLs (Kruglyak & Lander 1995), Bayesian mapping of QTLs (Satagopan et al. 1996, Sillanpää & Arjas 1998) and methods for differentiating pleiotropy from close linkage (Lebreton et al. 1998).

1.2.5.3 Conclusions from QTL mapping experiments

In the traditional models of quantitative genetics simplifying assumptions were made about equality and strict additivity of gene effects (Falconer & Mackay 1996). From the results of the QTL mapping experiments it has become clear that such assumptions are incorrect. In many mapping experiments, a relatively small number of QTLs accounts for very large portions of phenotypic variance, with increasing numbers of genes accounting for progressively smaller portions of variance, until the significance threshold is reached (Paterson 1995). The number of QTLs located for particular traits in individual studies varies from one to sixteen, usually being below five (Kearsey & Farquhar 1998). Up to four QTLs affecting one trait have been located on the same chromosome in barley (Tinker & Mather 1994). The proportion of phenotypic variation explained by each QTL and all QTLs together depends on heritability of the trait as well as on the portion of revealed QTLs. Individual QTLs may explain from 1 to 82 % of the phenotypic variation in each trait in barley (Barua et al. 1993, Yin et al. 1999). QTLs are usually spread over all

chromosomes, but clusters of QTLs in certain chromosomal regions have been observed as well. QTLs affecting several traits are common (Hayes et al. 1997) and may be due to pleiotropy or close linkage. Differences occur in QTL incidence when quantitative traits are scored in many environments or during many years. It looks like there are only a few QTLs with general influence and more with specific influence (Backes et al. 1995). In a study of barley malting quality a total of 184 QTLs were detected, but only 28 of these were observed in more than one environment (Thomas et al. 1996). However, comparative studies between related species have revealed conservation not only in marker order but also in locations of some QTLs (Lin et al. 1995).

Examples of QTL studies for different traits in various mapping crosses of barley are shown in Table 2. Markers associated with qualitative or quantitative resistance genes in barley are listed in Table 3.

Table 2. Examples of mapped quantitative trait loci in barley. The number of QTLs and the proportion of phenotypic variance explained by all or individual QTLs are shown.

Cross	**Trait**	**Number of QTLs**	**Phenotypic variance explained by all QTLs**	**Phenotypic variance explained by indiv. QTLs**	**Reference**
Steptoe x Morex	Heading date Height Yield Lodging Grain protein Alfa-amylase Diastatic power Malt extract Wort protein Malt beta-glucan Starch granule traits Dormancy	9 10 6 6 6 9 9 7 9 9 1-4 4	67% 72% 58% 71% 56% 63% 67% 57%		Hayes et al. 1993 Han & Ullrich 1994 Borém et al. 1999 Oberthur et al. 1995
Igri x Danilo	Stem breaking Ear breaking Kernel length Kernel shape Kernel weight	4 3 2 1 2	33% * 44% * 11% * 5% * 15% *		Backes et al. 1995

Table 2. cont.

Cross	Trait	Number of QTLs	Phenotypic variance explained by all QTLs	Phenotypic variance explained by indiv. QTLs	Reference
Harrington x TR306	Heading date Maturity Height Lodging Thousand grain weight Grain weight per volume Yield Grading Fine-grind extract Coarse-grind extr. Grain protein Wort protein Malt beta-glucan Alfa-amylase Diastatic power	9 5 9 6 9 5 5 5 6 4 6 5 3 3 7			Tinker et al. 1994
Morex x Dictoo	Winter survival Heading date	1 6	 66%	31-79% 11-20%	Pan et al. 1994
Tystofte Prentice x Volfsonger Gold	Straw length Lenth of top internode Length of basal internode Harvest index Total N in grain Total N in straw Total P in grain Total P in straw	2 3 2 3 2 2 3 2	38-63% 47% 62% 61-66% 20% 40% 39% 15-28%		Kjær et al. 1995 Kjær & Jensen 1995
Prisma x Apex	Preflowering duration Postflowering duration Leaf N content Specific leaf area Relative growth rate of leaf area	3-7 1-4 3-4 3 1		1-72% 8-18% 11-32% 15-57% 9%	Yin et al. 1999
Blenheim x E224/3	Germinative energy Germinative capacity Grain N content Milling energy Milling energy loss during malting	13 12 8 9 15	37-74% 35-81% 46-91% 20-76% 50-65%		Thomas et al. 1996
Tadmor x Er/Apm	Relative water content Osmotic potential Osmotic adjustment	3 4 1		6-8% 11-24% 18%	Teulat et al. 1998

Table 2. cont.

Cross	Trait	Number of QTLs	Phenotypic variance explained by all QTLs	Phenotypic variance explained by indiv. QTLs	Reference
Lina x HS92	/^{13}C content in control /^{13}C content in salt treated /^{15}N content in control /^{15}N content in salt treated Total N in control Total N in salt tr.	1 9 10 3 8 7	14% 80% 73% 27% 60% 69%		Ellis et al. 1997b
Clipper x Sahara	Boron tolerance	4			Jefferies et al. 1999

* Proportion of genetic variance explained

Table 3. Qualitative and quantitative resistance genes mapped or tagged with molecular markers in barley

Disease	Source of resistance	Resistance gene	Chromosome	Closest marker	Reference
Viral diseases					
BaMMV	10247 Bulgarian Russia 57	Ym8 Ym9 Ym11	4H 4H 4H	RFLP RFLP RAPD	Bauer et al. 1997
BaMMV/ BaYMV	Franka Ragusa Res.Ym No1	Ym4 Ym4 Rym5	3H 3H 3H	RFLP RAPD STS (RFLP) SSR CAPS	Weyen et al. 1996 Bauer & Graner 1995 Graner et al. 1999
BYDM	Ethiopian b. Shannon	Yd2 Yd2	3H 3H	STS (AFLP) CAPS	Paltridge et al. 1998 Ford et al. 1998
Fungal diseases					
Stem rust	Chevron	Rgp1	7H	STS (RAPD)	Horvath et al.1995
Barley leaf rust	Q21861 Q21861 Vada *H. v. spont.*	PphQ PphQ 6 QTL Rph16	 5H all except 1H and 3H 2H	RAPD STS (RFLP) AFLP STS (RFLP)	Poulsen et al.1995 Borovkova et al. 1997 Qi et al. 1998 Ivandic et al. 1999
Barley stripe rust	ICARDA/ CIMMYT line	2 QTL	5H 4H	RFLP RFLP	Chen et al. 1994

Table 3. (cont.)

Disease	Source of resistance	Resistance gene	Chromosome	Closest marker	Reference
Fungal diseases					
Barley powdery mildew	G. Zweiz.	Mlo	4H	RFLP	Hinze et al. 1991
	Ingrid NIL	Mlg	4H	RFLP	Görg et al.1993
	Pallas NIL	Mla	1H	RFLP	Jahoor et al.1993
	Vada	MlLa	2H	RFLP	Giese et al. 1993
	H. v. spont.	Mlt	7H	RFLP	Schönfeld et al. 1996
		Mlf	7H	RFLP	
		Mlj	5H	RFLP	Simons et
	Ingrid NIL	Mlo	4H	AFLP	al.1997
Scald	E224/3	Rh4, Rh10	3H	RFLP RAPD	Barua et al. 1993
	H. v. spont.	Rrs13	6H	RFLP	Abbott et al. 1995
	Atlas	Rh2	1H	RFLP	Schweizer et al. 1995
	Triton	Rh	3H	STS (RFLP)	Graner & Tekauz 1996
	H. v. spont.			RAPD	Hakim 1996
Net blotch	Steptoe/Morex	7 QTLs	all except 1H	RFLP	Steffenson et al. 1996
	Igri	Pt,,a	3H	RFLP	Graner et al. 1996
	Harrington/ TR306	4 QTLs	4H, 5H, 6H, 7H	RFLP	Spaner et al. 1998
	Arena/ Hor9088	12 QTLs	all except 5H and 7H	AFLP	Richter et al. 1998
	Galleon	Rpt4	7H	RFLP	Williams et al. 1999
Spot blotch	Steptoe/Morex	2 QTLs	1H and 7H	RFLP	Steffenson et al. 1996
Barley leaf stripe	Proctor	QTL	7H 2H	RFLP	Pecchioni et al. 1996
Other					
Bacterial leaf streak	Morex	2 QTLs	3H	RFLP RFLP	El Attari et al. 1998
Aphids	TR306	QTL	7H	RFLP	Moharramipour et al. 1997
CCN	Sahara 3771 Chebec	Ha2	2H	RFLP	Kretschmer et al. 1997
	Galleon	Ha4	5H	RFLP	Barr et al. 1998

1.2.6 Marker assisted selection

Marker assisted selection (MAS) is an indirect selection method relying on markers outside the target gene. Selection is not done based on the phenotype but based on a genotype of a marker that is linked to the gene affecting the phenotype. In theory, MAS is more effective than phenotypic selection when correlation between the marker genotype scores and the phenotypic values is greater than the square root of heritability of the trait, assuming that the heritability of the marker is 1 (Dudley 1993). MAS makes early selection before phenotypic evaluation possible and simplifies selection of traits that are difficult to score. Several requirements must be fulfilled before markers can be used in selection: close linkage between marker and the target gene, segregation for both the marker and the target gene, linkage disequilibrium in the plant population to be selected and a known linkage phase between the marker and the target gene (Weber & Wricke 1994). The efficiency of MAS can be increased by using markers flanking the target gene instead of a single linked marker (Tanksley 1983).

1.2.6.1 Introgression

Backcrossing is an approach to introgressing target loci from unadapted germplasm in to advanced genetic backgrounds. The backcrossing procedure is appropriate for traits controlled by a small number of loci. As the number of loci segregating for the trait increases, the number of backcross individuals which must be grown to have a high probability of recovering all favourable alleles also increases (Dudley 1993). Molecular markers can be used effectively to speed up and improve the precision of backcrossing. Firstly, markers linked to the target gene can be used to monitor the incorporation of the desirable alleles from the donor source (Tanksley 1983). Without markers it may be difficult to recognize individuals that carry the favoured allele among the backcross progeny because of low heritability, poor penetrance or the allele being recessive. Single-copy markers with defined map locations, such as RFLPs or SSRs, are ideal for the 'foreground selection' step (Toojinda et al. 1998). Secondly, selection for the molecular marker alleles of the recurrent parent can be used to speed up the recovery of the recurrent parent genotype (Young & Tanksley 1989). Markers with higher information content per reaction, such as AFLPs, are ideal for this 'background selection' step (Toojinda et al. 1998). Thirdly, linkage drag in the vicinity of the introgressed segment can be reduced by selecting for recurrent alleles at loci linked to the target gene. Young and Tanksley (1989) estimated that an introgressed segment could be reduced in two generations, by MAS for recurrent parent genotype, to a size that would require 100 generations without MAS.

Marker assisted backcrossing is useful for rapid transfer of resistance genes from wild progenitors to advanced breeding lines. Pyramiding of several resistance genes into a single genome could be greatly enhanced with molecular markers (Melchinger 1990). A new application of MAS is in backcrossing of transgenes from model varieties amenable to transformation to the most advanced germplasm as quickly as possible (Lee 1995). The

disadvantage of marker assisted backcrossing is that positive factors for traits unrelated to the the main objective will be eliminated as well as the negative factors associated with the donor parent.

Manipulation of QTLs in backcross breeding programs differs slightly from that of qualitative traits. The segregation of a single QTL can be observed only through the linked marker, not directly from the phenotype. Since QTL locations are usually estimated imprecisely, the chromosomal segment to be transferred and followed with flanking markers should be at least 10-20 cM long (Visscher et al. 1996). According to simulation studies, it is possible to manipulate up to four unlinked QTLs simultaneously with population sizes of a few hundred, assuming optimally positioned markers (Hospital & Charcosset 1997). Backcrossing of QTLs can be problematic due to loss of target loci through recombination, incorrect information regarding the location of the QTLs and negatively altered expression of the QTLs in new genetic backgrounds (Toojinda et al. 1998). MAS has been successfully used in barley to introgress two stripe resistance QTLs through backcrossing to a genetic background different from the one used for QTL detection (Toojinda et al. 1998).

1.2.6.2 Line development

In breeding autogamous species lines are developed from crossing schemes including two or more parents. In a backcross programme a few traits would be transferred from a donor to a recipient. In line development, however, good characteristics from all parents should be combined in a single line (Weber & Wricke 1994). Information on mapped QTLs can be used to design matings that maximize the probability of pyramiding most, if not all, favourable QTL alleles in a single genotype (Dudley 1993). For traits with significant interactions between QTLs emphasis should be placed on identification of the best multi-locus allelic combinations instead of simply collecting many alleles with positive effects (Zhu et al. 1999).

The relative efficacies of MAS and traditional selection for improving quantitative traits have been considered in several simulation studies. As reviewed by Lee (1995), the efficiency of MAS is enhanced and may be more efficient than traditional selection under the following circumstances: 1) the trait under selection has low heritability; 2) tight linkage between QTL and markers (<5cM); 3) in earlier generations of selection prior to fixation of alleles at or near marker loci and recombinational erosion of marker-QTL associations; 4) large sample sizes for mapping and selecting QTL are used to improve estimates of QTL alleles. Markers very closely linked to the target genes or even located in the gene can greatly enhance the use of MAS in advanced generations, where the linkage disequilibrium becomes smaller. The accurate chromosomal locations of QTLs, as well as the magnitude of QTL effects, should be verified prior to their use in an applied breeding program. In barley, the effect of four yield QTLs was verified using a set of DH lines different from the lines used for mapping (Romagosa et al. 1999). In that study, selections

based on marker genotypes, or combined information from markers and phenotype, were at least as efficient as phenotypic selection alone, but qualitative QTL x E interactions decreased the efficiency of MAS for some of the QTLs. In the same barley lines, effects of only one of the two major QTL regions for several malting quality traits were verified, the effects of the other region were lost probably due to inaccurate location of the QTL (Han et al. 1997).

Simultaneous selection for multiple traits complicates the use of MAS in breeding. Information on several markers needs to be combined when selection is made. One method is to determine the marker genotype of each line being tested and sum the significant additive effects of each marker locus to an index value (Dudley 1997). A large number of plants have to be scored in order to find the desired marker combination in the progeny, which may render the selection procedure costly (Graner 1996).

1.2.7 Map-based cloning

Map-based or positional cloning offers a possibility to clone a gene despite the lack of information regarding the corresponding gene product. The original concept behind map-based cloning was to find a DNA marker linked to a gene of interest, and then walk to the gene via overlapping clones (cosmids or yeast artificial chromosomes, YACs) (Wicking & Williamson 1991). Chromosome walking in complex plant genomes is hampered both by the large amounts of DNA being traversed and by the prevalence of repetitive DNA (Tanksley et al. 1995).

Development of efficient marker technology and genetic screening methods avoid these problems by identifying one or more DNA markers at a physical distance from the target gene that is less than the average insert size of the genomic library being used for clone isolation. This approach, termed chromosome landing (Tanksley et al. 1995), includes mapping of the target gene on a restricted area of a chromosome, confirming the gene location for example with marker assisted introgression, fine mapping the area using for example BSA and NILs, and selecting YAC and BAC clones with markers closely linked to the target locus. Chromosome landing has been used for isolating and sequencing the powdery mildew resistance genes *mlo* (Büschges et al. 1997) and *Mla* (Wei et al. 1999) in barley. The synteny between rice and barley has been used to saturate the region containing the stem rust resistance genes, with molecular markers (Kilian et al. 1997). Chromosome landing has also been used for identifying YAC clones encompassing the barley *Rar1* gene, which is involved in the powdery mildew defence response (Lahaye et al. 1998).

1.2.8 From structural genomics to functional genomics

Structural genomics involves genetic mapping with molecular or visible markers as well as physical mapping in which YACs and bacterial artificial chromosomes are aligned with the chromosomes (Terryn et al. 1999). Genetic maps exist for many agriculturally important plants, but physical maps only for a limited number of crops. Sequencing the entire *Arabidopsis thaliana* (L.) genome, the size of which is only 120 Mb, is estimated to be completed within a few years (Bouchez & Höfte 1998). Genomic sequencing has also been started with rice, which has a small genome size of 430 Mb and serves as a model species for monocotyledonous species. In barley, as in many other crops with a larger genome, sequencing has been concentrated on ESTs - single sequence reads of randomly selected cDNA clones. The International Triticeae EST Co-operative (ITEC) has sequenced 12 500 ESTs from barley (http://wheat.pw.usda.gov/genome). The goal of ITEC is to have 40 000 EST sequences publicly available in July 2000 and later to have as many as 300 000 ESTs sequenced.

The structure and function of genes in a genomic sequence can still only be predicted with great difficulty (Terryn et al. 1999). Knowing when and where a gene product is expressed can provide important clues to its biological function. Large scale monitoring of gene expression is greatly enhanced by differential display methods (Liang & Pardee 1992) and especially with DNA micro-arrays (Desprez et al. 1998). DNA microarrays may be used as a kind of 'reverse Northern blot' whereby DNA clones or PCR-generated fragments are spotted in a dense array and hybridized to RNA-derived probes (Terryn et al. 1999). Micro-array analysis provides a way to link genomic sequence information and functional analysis and will produce enormous amounts of data for genome analysis in plants in the near future. A challenge in the next decade will be to build integrated databases combining information on such things as sequence, map position, mRNA and protein expression, mutant phenotypes, metabolism and allelic variation (Bouchez & Höfte 1998).

1.3 Aims of the study

The aim of this study was to develop DNA markers for barley breeding.

The specific aims were:

- To assess the genetic diversity within Finnish six-rowed barley germplasm.
- To construct a linkage map based on Finnish six-rowed breeding lines.
- To study whether distorted segregation is caused by genes affecting anther culture response.
- To find markers linked to specific agronomically important traits for use in marker-assisted-selection in barley breeding.
- To dissect the quantitative variation observed in the agronomical traits within the six-rowed barley breeding material using QTL mapping.

2. Material and methods

2.1 Plant material

For genetic mapping, doubled haploid progenies were produced by anther culture (Manninen 1997) from the F_1 generation of three barley crosses:

a) Ingrid (*mlo11*) x Pokko

An isogenic line of Ingrid, carrying the *mlo11* allele, was used in the cross as the donor of resistance to *Erysiphe gramines* DC. ex Mérat *hordei* Marchal. Sixty doubled haploid progeny lines were used for DNA-analysis and detection of markers linked to the *Mlo* locus (III).

b) Rolfi x CI9819

An ethiopian two-rowed barley line CI9819 conferring resistance to a wide range of *Pyrenophora teres* Drechs. f. *teres* Smedeg. isolates was crossed with Rolfi, a six-rowed Finnish variety susceptible to net blotch. Hundred and nineteen doubled haploid lines were used for DNA-analysis and mapping of net blotch resistance genes (IV). F_1, F_2 and BC_1 progenies were tested for their disease reaction in addition.

c) Rolfi x Botnia

Two hundred and three doubled haploid lines were produced from a cross between doubled haploidized Rolfi and Botnia. Rolfi and Botnia are both Finnish six-rowed spring barley varieties. Rolfi is an early, high yielding barley for feed released in 1997, and Botnia is a later, high yielding barley released in 1996 for ethanol and starch production and for malting. 190 DH-lines from this cross were used for mapping QTLs affecting agronomic performance (V). In addition, a selection of 31 DH lines were used to detect associations between marker genotype and anther culture response (II).

In addition to the mapping crosses, several barley genotypes were used. In paper III, four varieties resistant to powdery mildew (Salome, Apex, Chariot, Verner) and five susceptible ones (Prisma, Triumph, Kustaa, Pokko, Ingrid) as well as Ingrid isogenic lines carrying the *mlo1-mlo11* alleles were studied with DNA-markers linked to the *Mlo* locus. All isogenic lines of Ingrid were kindly provided by Prof. J. MacKey, Swedish University of Agricultural Sciences.

Genetic variation was studied in a collection of modern barley genotypes (Botnia, Rolfi (=Jo1632), Pohto, Arve, Hja85194, Erkki (=Hja87061), old genotypes (Olli, Gull, Asplund, Vega, Hannchen, Maskin) and three genotypes considered to be foreign introgressions into the Nordic barley genepool (OAC21, Hiproly, Andie). Both United States Department of Agriculture and the Nordic Gene Bank supplied seed samples for this study (I).

Barley-wheat addition lines obtained from B.S. Gill (Kansas State University, USA) were used for locating markers to specific chromosome arms (IV).

2.2 DNA-analyses

2.2.1 DNA extraction

The DNA of the barley plants was extracted from foliage of 10-14 day old seedlings grown in a greenhouse. A modified CTAB-method was used (Poulsen et al. 1993), with the exceptions that CsCl density gradient centrifuging was omitted and a Rnase treatment (1µg RNase/1ml diluted DNA, 30 min. at 37°C) was added. DNA concentrations were determined using a GeneQuant II RNA/DNA Calculator (Amersham Pharmacia Biotech) and all samples were diluted to a concentration of 0.5 mg/ml and stored at -20°C. In paper III small-scale isolation of DNA (Tinker et al. 1993) and a simple squash method (Langridge et al. 1991) were used as well.

2.2.2 RAPDs

Decamer primers for RAPDs were either purchased from Operon Technologies (Alameda, California, USA) or synthesized on an Applied Biosystems 392 DNA/RNA Synthesizer. RAPD bands were named according to their approximate molecular weight with prefix OP marking fragments amplified with the Operon primers (e.g. OPA12-850 is a 850 bp fragment amplified with the Operon A12 primer). DNA amplification for RAPDs and separation of the amplified fragments was performed as described in papers I and III.

2.2.3 RFLPs

Before RFLP analysis DNA samples were run on agarose gels to confirm that DNA was not cleaved. EcoRI, EcoRV, HindIII and XbaI were used for digestion of DNA. Digestion reactions included 8µg barley DNA and 40 U of restriction enzyme in 40µl of 1x buffer supplied for each enzyme. Digestions were kept overnight at 37°C and then loaded on 0.8% agarose gels and run in electroforesis (50V, overnight). The gels were first rinsed for 30 min in denaturation buffer (0.5 M NaOH, 1.5 M NaCl), briefly in distilled water and then neutralized for 2 x 15 min (1.5 M NaCl, 0.5M Tris-HCl pH 7.2, 0.001M EDTA). Southern blotting and hybridization were made on Hybond N+ membranes (Amersham Pharmacia Biotech) according to the manufacturer's instructions (version 2). Both genomic and cDNA clones were used as probes. Probes were kind gifts from A. Graner, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (barley clones: MWG,

cMWG), M. Sorrells, Cornell University, Ithaca, NY, USA (barley clones: BCD, oat clones: CDO, wheat clones: WG) and A. Kleinhofs, Washington State University, Pullman, WA, USA (barley clones: ABC, ABG, *Triticum tauschii* clones: KSU). Clones were amplified in PCR with suitable primers and purified with Wizard PCR Preps columns (Promega) before use. Radioactive labelling (^{32}P) and autoradiography were used to visualize the RFLPs.

2.2.4 Microsatellites or SSRs

Microsatellite primers described previously (Liu & Somerville 1996, Becker et al. 1995, Russell et al. 1997) were used. Primers were synthesized on an Applied Biosystems 392 DNA/RNA Synthesizer, one primer of each primer pair labelled with a fluorecein label. Amplification conditions for microsatellites were described in papers II and IV. Microsatellite products were resolved with an ALF DNA sequencer and analyzed with the Fragment Manager computer program (Amersham Pharmacia Biotech).

2.2.5 REMAPs, IRAPs and ISSRs

Amplification of REMAPs, IRAPs (inter-retrotransposon amplified polymorphism) and ISSRs (inter-simple sequence repeat amplified polymorphism) is described in detail in paper IV.

2.3 Evaluation of traits

2.3.1 Disease tests

Disease tests were performed in greenhouse conditions and symptoms were scored visually. Inoculation of barley plants with powdery mildew is described in paper III. Net blotch isolates and inoculation methods used were explained in paper IV.

2.3.2 Anther culture and ploidy determination

Thirty one selected lines, together with the parental varieties Rolfi and Botnia, were tested for their anther-culture response. Anthers were cultured according to the best method established by Manninen (1997). Experimental details were described in paper II. Ploidy level of the regenerants was determined using flow cytometry as described by Rokka et al. (1995).

2.3.3 Agronomical traits

Agronomical traits were evaluated in unreplicated field experiments conducted at 1-3 sites during 1995-1997 (V). Heading date and days to maturity were observed in the field and later transformed to accumulated effective degree days from sowing to heading and accumulated effective degree days from heading to maturity. Seven other traits were evaluated as described in paper V.

2.4 Statistical methods

All calculations and statistical analyses used were described in each paper. Both parametric (IV, V) and non-parametric (II) methods were used for analyzing the data. Linkage maps were constructed using either Mapmaker/Exp 3.0 (Lander et al. 1987) (III) or JoinMap 2.0 (Stam & Van Ooijen 1995) (II, IV) programs. Genetic mapping of traits (IV, V) was done by MQTL 0.98 (Tinker & Mather 1995b). Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) were used. Genome-wide error rates (5%) for the test statistics (Haley & Knott 1992) were calculated using permutation tests (Churchill & Doerge 1994).

3. Results

3.1 Diversity of Finnish six-rowed barley (I)

Genetic diversity among Finnish six-rowed spring barleys was studied using both pedigree analysis and RAPD markers. Finnish plant breeders released 26 six-rowed spring barley varieties up until 1996, 22 of which originate from controlled crosses. According to their pedigrees, these varieties are largely composed of seven ancestors, namely Olli, Asplund, Vega, Gull, Hanna, Maskin and OAC21. The contribution of these principal ancestors to the pedigrees of the varieties has not changed significantly during the 60 years of barley breeding in Finland. Jaccard's similarity indices, based on 54 polymorphic RAPD markers, were calculated for a set of modern, ancestral and introgression lines and varieties. Jaccard's indices ranged from 0.154 to 0.935, and similarities between related accessions were significantly higher (*t*-test, $P<0.01$) than between the unrelated ones. However, Jaccard's indices up to 0.914 were detected among accessions assumed, according to pedigree information, to be unrelated. The genetic similarity revealed by RAPD markers within the old varieties was comparable to that of modern varieties. Correlation between kinship coefficients based on pedigrees and Jaccard's indices based on RAPD data was very weak ($r=0.14$, $P>0.05$).

3.2 Linkage maps of the barley genome (II, IV)

Two linkage maps covering the barley genome were generated in these studies. For the first one a narrow-based cross between two six-rowed spring barley varieties Rolfi and Botnia was used (II) and for the second one, a wider cross between Rolfi and an Ethiopian line CI9819 was used (IV). The degree of difference detected by DNA-markers was approximately three times higher in the second cross. Both maps were based on doubled haploids produced from the F_1 generation by anther culture. For 22.5% of markers in the Rolfi x Botnia cross and for 29.0% of the markers in the Rolfi x CI9819 cross segregation was distorted. In the former cross the majority of the skewed markers were located on chromosomes 3H and 7H and in the latter on 2H, 3H and 5H. The positions of the anchor markers in the Rolfi x Botnia and Rolfi x CI9819 maps in relation to the consensus barley map are shown in Fig. 1.

3.2.1 Rolfi x Botnia map

To avoid problems caused by heterogenity or heterozygosity in the parents, the parental genotypes were doubled haploidized before crossing. Two hundred and three doubled

haploid progeny lines were produced from F_1 by anther culture and analyzed with three types of molecular markers (RAPDs, RFLPs and microsatellites).

The parents of the doubled haploid progeny differed in 28 of 180 (15.6%) clone enzyme combinations tested; 14 RFLP loci were mapped based on these differences. 104 of 1275 (8%) amplified RAPD bands and 3 of 17 (18%) amplified microsatellites differed between Rolfi and Botnia. In the doubled haploid progeny lines the Rolfi alleles were over-represented when all the 111 loci were considered together. The percentage of marker loci with Rolfi alleles in each doubled haploid line varied between 17-100% and 8 lines had exactly the same marker profile as Rolfi. Three doubled haploid lines were not completely homozygous but heterozygosity was detected in two to three RFLP loci. This may be caused by fusion of gametes before anther culture or outcrossing during seed propagation. These lines were not used for further analysis.

The results of linkage analyses done with two computer programs, Mapmaker/Exp 3.0 and JoinMap 2.0 were compared. Markers were first grouped with LOD thresholds in the range 3.0 – 7.0 using 0.5 LOD step size. The recombination fraction was not limited (0.5). Spurious linkages between anchor markers (RFLP, microsatellites) were detected with both programmes at the lower LOD thresholds, but at the LOD 5.0 JoinMap grouped the markers correctly. With the same LOD threshold Mapmaker still linked three linkage groups together; all of these groups having Rolfi alleles in excess. This spurious linkage could easily be avoided by either setting the LOD threshold to 5.5 or by restricting the recombination fraction to 0.3.

JoinMap was used for map construction. Markers were first grouped using a 5.0 LOD threshold and afterwards groups and unlinked anchor markers were joined to form chromosomes based on previous information on anchor-marker locations. Eighty five markers were mapped to linkage groups keeping the jump threshold at 3.0 in JoinMap. A reliable position could not be found for 19 RAPD markers although they were assigned to chromosomes. Markers tended to cluster to certain areas of the genome leaving others uncovered. Six markers mapped exactly to the same position on chromosome 3H. The Rolfi x Botnia linkage map included 85 DNA markers and covered 654 cM. Only one polymorphic marker was assigned to chromosome 1H and large gaps exist on the maps of other chromosomes.

3.2.2 Rolfi x CI9819 map

The second map was based on a cross between Rolfi and an Ethiopian barley line CI9819, which was resistant to net blotch. Six different types of markers, RFLP, RAPD, REMAP, IRAP, ISSR and microsatellites, were applied to the mapping population. In RFLP analysis, 74 clone and enzyme combinations were tested and 32 (43%) established a difference between the parents. Twenty-nine microsatellite loci were screened and 16

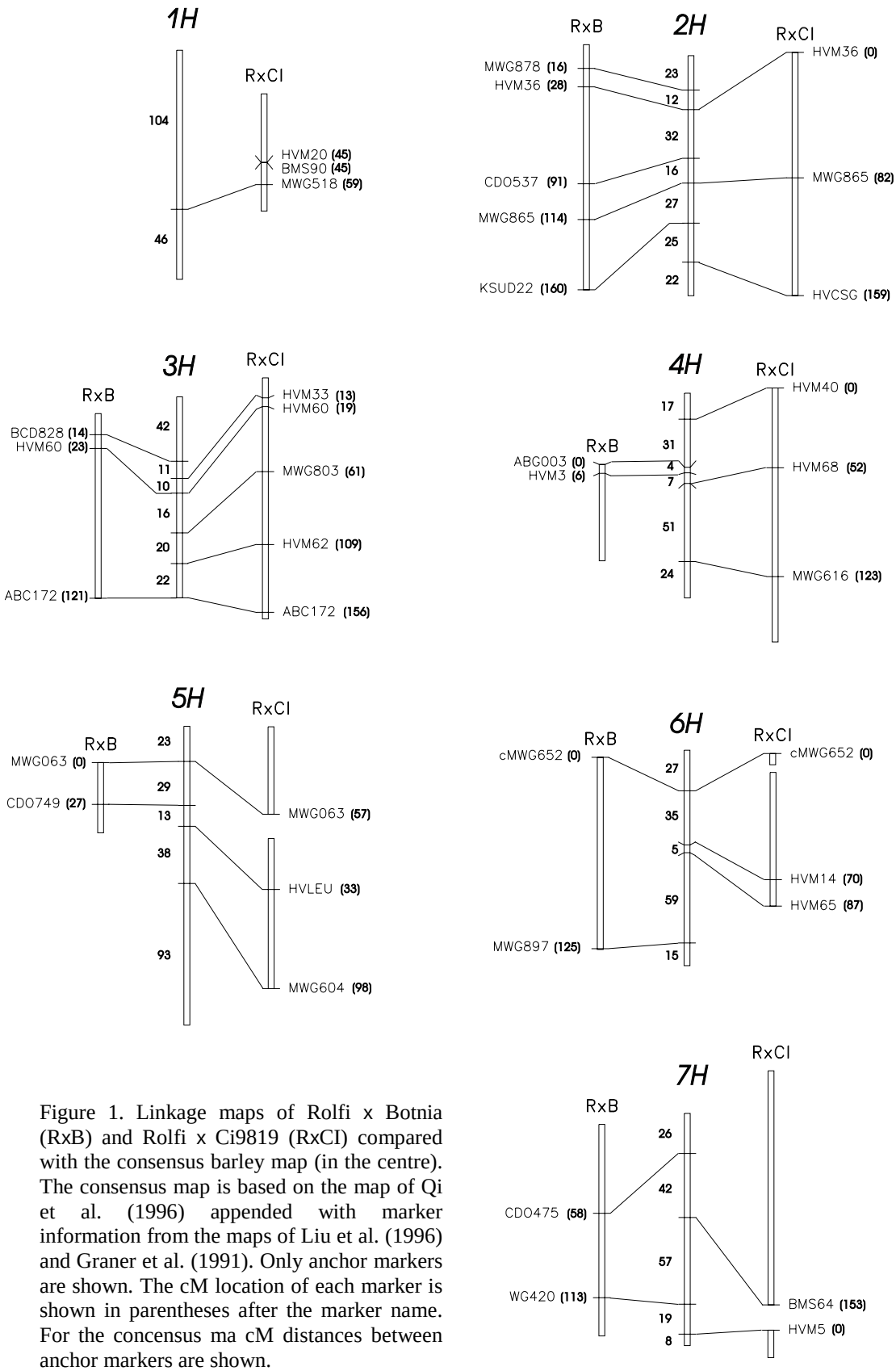

Figure 1. Linkage maps of Rolfi x Botnia (RxB) and Rolfi x Ci9819 (RxCI) compared with the consensus barley map (in the centre). The consensus map is based on the map of Qi et al. (1996) appended with marker information from the maps of Liu et al. (1996) and Graner et al. (1991). Only anchor markers are shown. The cM location of each marker is shown in parentheses after the marker name. For the concensus ma cM distances between anchor markers are shown.

polymorphic ones (55%) were found. From the 283 RAPD primers tested, 171 (60%) found at least one difference between the parents; together these 171 primers revealed 303 band differences between the parents. The REMAP reactions generated 20 to 40 bands, the product lengths varying from 100 to 3000 bp. For mapping, 215 polymorphic REMAP bands (~ 9 bands per primer combination) were chosen.

Altogether 322 markers were analyzed in the progeny and 306 (215 REMAPs, 39 ISSRs, 24 IRAPs, 15 microsatellites, 8 RFLPs, and 5 RAPDs) were placed on the linkage map. A LOD score as high as 9.5 was used for constructing the linkage groups; with lower LOD thresholds, anchor markers from different chromosomes were grouped together. Twenty linkage groups were formed and 13 of these were assigned to chromosomes. All of the unassigned linkage groups except one contained three or fewer markers. In total 29 markers could not be assigned to any of the chromosomes.

The linkage map covers 1016 cM (an average of 6.5 cM between each locus) of the barley genome and all seven chromosomes are represented. Dense clusters of markers were observed on chromosomes 2H, 6H, and 7H and gaps longer than 30 cM were present on chromosomes 3H, 4H, 5H, and 7H.

3.3 Associations between anther culture response and DNA markers (II)

The segregation of 111 molecular markers was tested in 200 doubled haploid progeny from the Rolfi x Botnia cross and, with a subset of markers, was compared with segregation in F_2 progeny from the same cross. In addition, a selection of 31 doubled haploid progeny lines were tested for their anther-culture ability (anther response, plant regeneration, green plant ratio and spontaneous diploidization) to locate genes controlling anther-culture response in barley.

Of the 111 markers analysed in the doubled haploid progeny 25 loci (22.5%) showed distorted segregation ($P \leq 0.00045$). The majority of these loci were located on chromosome 3H and 7H. For comparison of segregation a subset of 25 RAPD markers were tested in the F_2 from the same cross. Only one marker had a skewed segregation and it was located in a region not distorted in the doubled haploid progeny.

Although Rolfi and Botnia were both recalcitrant in anther culture, they differed significantly for anther response and regeneration rate per number of responsive anthers. In the doubled haploid progeny the effect of the genotype was highly significant for the number of responsive anthers, number of plants per responsive anther and the proportion of green plants ($P<0.001$, Kruskal-Wallis test). When the effect of each marker allele on these parameters was tested with a selection of 36 evenly distributed markers, mean ranks for each line were used and the percentages of diploid green plants were included in the analysis. When the Bonferroni corrected significance threshold ($P \leq 0.00035$) for a repeated

Mann-Whitney U-test was used, only one marker-trait association was found, namely between ABG003 and anther response on chromosome 4H. When a more relaxed significance threshold ($P<0.05$) was used, ten markers were associated with anther culturability. Two chromosomal regions associated with anther response were found on chromosomes 2H and 4H, the Botnia alleles being associated with the favourable effect in both cases. Three regions were associated with plant regeneration on chromosomes 2H (two regions) and 3H, Rolfi alleles being favourable in two and Botnia alleles in one of the cases. Rolfi was the parent with higher rates of regeneration. One marker associated with diploidization rate was found on chromosome 4H. No association could be found between the significantly skewed markers on 7H and any of the anther culture traits. A chromosomal region contributing to anther culture traits was found on 4H, which segregated in a Mendelian fashion in the doubled haploid progeny. On chromosome 4H the selection pressures caused by anther culture may have been in opposite directions since Botnia alleles were favourable for anther response and Rolfi alleles for spontaneous diploidization.

3.4 Tagging and mapping of disease resistance genes (III, IV)

3.4.1 Tagging the powdery mildew resistance locus, *Mlo*, with RAPD markers

Bulked segregation analysis was used in order to find RAPD markers linked to the *Mlo* locus on chromosome 4H. Seven markers showed linkage to the *Mlo* locus when analysed in the 60 doubled haploid progeny lines and a partial map of chromosome 4H could be constructed. The closest marker, OPF4-980, was located less than 2 cM from the *Mlo* locus. Three of the linked markers were previously mapped in Rolfi x Botnia cross to chromosome 4H. When Ingrid and eleven isogenic lines, each carring a different *mlo* allele, were studied, the closest marker was amplified only from the Ingrid *mlo*11 isogenic line. Consequently, this amplified fragment must originate from the same source as the *mlo*11. Markers more distantly (;10 cM) linked to *mlo*11 were also amplified in Ingrid. When resistant and susceptible varieties were studied, only the resistant varieties showed the OPF4-980 marker.

3.4.2 Mapping of net blotch resistance genes

Two loci affecting seedling resistance to net blotch in barley were mapped in the Rolfi x CI9819 cross with the aid of molecular markers. A major resistance gene accounting for 65% of phenotypic variation was located by both SIM (TS=120.4) and sCIM (TS=165.1) at the microsatellite marker BMS18 on chromosome 6H. The 30 cM segment that had a significant effect on resistance contains 26 markers. Based on the small infection response values scored for the F_1 individuals, the major resistance gene on 6H was dominant. An

epistatic locus having a smaller effect was found on chromosome 5H. In both of the loci affecting resistance, CI9819 alleles increased resistance to net blotch. At the epistatic locus this effect was detected only when the 6H resistance locus possessed Rolfi alleles. The same region on 6H has been shown to control resistance to net blotch in two additional crosses (Rolfi x Rojo, Rolfi x Coast) (our unpublished results).

3.5 QTL analysis of agronomical traits (V)

The number, location and effect of QTLs affecting pre-heading and post-heading duration and several other important agronomic traits were determined in the Rolfi x Botnia cross. Experiments were conducted at one to three sites during three years to measure degree days required for heading (preH), degree days required from heading to maturity (postH), straw length (SL), ear length (EL), yield, thousand grain weight (TGW), hectolitre weight (HLW), grading >2.5mm (GR2.5) and number of grains per ear (GPE). A map containing 71 DNA markers was used for QTL mapping, and 34 genetic factors associated with agronomic traits were detected. Three genetic factors affected preH, three postH, four SL, three yield, five HLW, seven TGW, six GR2.5, one GPE and two EL. QTLs were located on every chromosome except 1H, which was missing from the linkage map. Epistatic interactions for TGW and GR2.5 were noted. Both main effects and QTL x environment interaction were recorded for preH, postH, SL, TGW and GR2.5. Clustering of QTLs was observed especially on chromosomes 2H, 3H, 6H and 7H. Loci affecting pre-heading duration and post-heading duration were partly different. In addition, Rolfi alleles had opposite effects on these characters at the QTLs on 2H and 7H.

4. Discussion

Barley germplasm utilized in local barley breeding is often genetically narrow-based. A narrow genetic base seems to be a prerequisite for breeding elite high-yielding varieties (Rasmusson 1992). It may be that adaptation and high yield are based on co-adapted gene complexes (Allard 1988) and advantageous interactions among loci may be disrupted in wider crosses. However, breeders have expressed their concern about the narrow genetic base. Lack of variability may lead to vulnerability, if, for example, new virulent pathogen races evolve. It is also clear that the adapted, elite germplasm does not contain all the most desirable alleles available in a species.

Coancestry based on pedigree information and genetic similarity based on DNA markers represent different approaches to determine diversity in barley. Several authors have found from low to moderate correlations between RFLP-based (Graner et al. 1994, Casas et al. 1998), RAPD-based (Tinker et al. 1993) and AFLP-based (Schut 1997) estimates of genetic similarity and coancestry in barley. Correlations are lower when the genotypes studied belong to the same breeding pool sharing many ancestors in their breeding history

(Graner et al. 1994), as is the case in this study. Coancestry coefficients are based on many simplifying assumptions: ancestors with unknown pedigrees being unrelated, both parents contributing equally to the offspring and all lines being homozygous and homogeneous (Graner et al. 1994). Contrary to these assumptions, the similarity of RAPD patterns in many of the ancestors for Finnish six-rowed varieties indicates that the ancestors are related. In addition, some Finnish six-rowed barley varieties are not totally homogeneous (Roininen et al. 1992). Violation of all the assumptions underlying pedigree analysis may lead to spurious coancestry coefficients.

The accuracy of genetic similarity estimates based on molecular markers depend on the number of markers, their distribution over the genome, nature of evolutionary mechanisms underlying the variation (Powell et al. 1996) and reliability of scoring. RAPDs were used for assessing genetic variability in this study. RAPDs are relatively easy to apply, and in an autogamous, and thus homozygous, species like barley, dominance of RAPDs poses no problem. The distribution of RAPD markers used was known only for ten markers, which were mapped in the Rolfi x Botnia cross on five different linkage groups. When linkage disequilibrium exists, as expected with the related accessions in this study, equally spaced markers would afford a better estimate than randomly distributed markers (Powell et al. 1996). Different markers give similarity estimates slightly differing from each other and rank accessions in specific ways (Russell et al. 1997) This may be due to different mechanisms underlying the generation of new variants in RAPD markers compared with RFLP and AFLP, which both detect mutations in the restriction sites (Powell et al. 1996).

Knowledge of genetic variation at DNA level can aid widening the genetic base of breeding lines. However, diversity of neutral molecular markers may not reflect diversity of quantitative traits (Karhu et al. 1996). Information on diversity of molecular markers would be more valuable to breeders, if markers linked to important QTLs were used in diversity analysis. Associations between AFLP polymorphism and QTL polymorphism were detected for a wide range of malting quality and agronomic traits in barley, when markers linked to genomic regions with significant QTL effects in several mapping crosses were used (Hayes et al. 1997). An integrated approach of genetic diversity analysis and QTL mapping could assist in mating design and exploitation of exotic germplasm. The net effect should be that plant breeders can maintain mean performance and at the same time maximize genetic diversity (Hayes et al. 1997).

Finnish six-rowed barley is genetically narrow-based. Most of the variability included in the Finnish landraces (Sauli 1927) has been lost in the early phases of barley breeding in Finland: only two accessions have had an impact on Finnish six-rowed barley breeding. In Finland barley is cultivated at the Northern species margin and the climatic conditions set strict limits to the barley germplasm that can be used for variety breeding. To study the genetic basis of agronomically important traits within the narrow-based elite Finnish barley germplasm, two six-rowed spring barleys, Rolfi and Botnia, were chosen as parents of a mapping cross. These varieties (or then lines Jo1632 and Jo1599) were considered to

represent well the agronomic and quality variation existing within the six-rowed barley breeding material. The coancestry coefficient of Rolfi and Botnia is 0.135 and the level of polymorphism in molecular markers was low. This was reflected in the construction of the linkage map: scarce markers were detected on chromosome 1H and several other segments of the barley genome. Difficulties in filling the gaps in a map may be due to chromosomal regions which are identical by descent in the parents of the cross (Graner 1996). The Rolfi x Botnia map was based mainly on RAPD markers. RAPD markers have been reported to map non-randomly in the barley chromosomes (Giese et al. 1994). This may explain, in part, the poor coverage of the Rolfi x Botnia map. However, when 17 anchor markers (RFLPs, microsatellites) were added to the map, 14 mapped to the areas already covered with RAPD markers. Eventhough the linkage map contained large gaps, it could be used for QTL analysis. A map with 50 cM marker spacings should be adequate for QTL detection (Darvasi & Soller 1994). Only two gaps exceeding 50 cM existed on the Rolfi x Botnia map.

The Rolfi x Botnia map was based on doubled haploid lines produced by anther culture. During the molecular analysis, it became clear that a strong deviation from the expected Mendelian segregation existed among the doubled haploid lines. Segregation distortion is a common phenomenon in anther culture derived barley (Heun et al. 1991, Graner et al. 1991, Becker et al. 1995, Zivy et al. 1992, Thompson et al. 1991). Markers with non-random assortment of alleles are not distributed at random throughout the genome but are restricted to particular areas on different chromosomes (Graner 1996). Genomic regions with most severe segregation distortion in the Rolfi x Botnia map coincide with regions with skewed marker segregation in other crosses (Barua et al. 1993, Heun et al. 1991, Graner et al. 1991, Becker et al. 1995). Anther culture is presumed to invoke selection on the random sample of gametes contained by the anthers and it has been suggested that the distorted genomic areas may contain genes affecting survival in anther culture (Barua et al. 1993, Zivy et al. 1992, Thompson et al. 1991). To study this, we examined anther culture response in a selection of Rolfi x Botnia doubled haploid lines. Associations between markers and anther culture response were detected, but not only in the distorted genomic regions. At some of the distorted regions no effects on anther culture response were found. Due to the small number of doubled haploid lines studied, it is unlikely that all loci affecting anther culturability and causing segregation distortion could be detected. In our doubled haploid progeny, the observed segregation distortion could be explained by genes affecting anther culture for chromosome 3H, but for the remainder of the distorted genomic regions there is no adequate explanation. Interestingly, two somatic shoot regeneration loci have earlier been located in the same positions on chromosomes 2H and 3H (Mano et al. 1996), where associations between markers and regeneration of plantlets was found in the Rolfi x Botnia cross. This may indicate, that at least some of the same genes are involved in plant regeneration from somatic and androgenetic tissues. The function of these genes *in vivo* remains to be solved.

Androgenetic doubled haploids are widely used in breeding and genetic studies of barley. Segregation distortion may have consequences for all applications of androgenetic doubled haploids including genetic linkage analysis. The most commonly used software in genetic map construction, Mapmaker (Lander et al. 1987), assumes undisturbed segregations and analysis may lead to spurious linkages if segregation is distorted (Foisset & Delourme 1996). JoinMap, on the other hand, uses LOD scores based on the chi-square test for independence of segregation and thus segregation distortion does not affect linkage analysis (Stam & Van Ooijen 1995). We used JoinMap for map construction in both Rolfi x Botnia and Rolfi x CI9819 crosses. Segregation distortion should not affect QTL mapping if the construction of linkage map has been accomplished properly (Van Ooijen, personal comm.).

In the narrow-based Rolfi x Botnia cross QTLs affecting important agronomic traits were located on each chromosome except 1H. From one to seven QTLs affecting each trait were detected; these numbers of QTLs should be treated as a minimum estimate. It is probable that only the largest effects were established and many small QTLs were not noted. Due to difficulties in detecting polymorphic markers, the linkage map used did not cover the entire barley genome and consequently some effective QTLs may have been left undetected. Many QTLs detected in the Rolfi x Botnia cross overlapped with previously found QTLs, especially those found in the North American germplasm (Hayes et al. 1993, Tinker & Mather 1994, Han & Ullrich 1994). Candidate loci were identified for QTLs affecting earliness and straw length in Rolfi x Botnia cross, namely the earliness per se locus *eps2S* (Laurie et al. 1995) and GA insensitive dwarfing gene *gai* (Börner et al. 1999). As suggested by (Robertson 1985), alleles with quantitative effects as well as alleles with qualitative effects may exist at the same locus.

In this study, consistent with earlier barley studies (Hayes et al. 1993, Kjær et al. 1995, Hayes et al. 1997, Tinker & Mather 1994), clustering of QTLs was detected. The coupled effects detected may be either due to close linkage or due to pleiotropic effects of a single gene. Since most of the QTL clusters in this study were located in the centromeric area of the chromosomes, clustering may be explained by the suppression of recombination in these regions (Pedersen & Linde-Laursen 1995, Tanksley et al. 1992).

QTL x E interactions can be utilized when breeding is aimed at specifically defined target localities. In this study, the experimental sites were selected to represent different soil types and climatic conditions in Finland. Still, the QTL x E interactions were usually of lesser importance than the main effects. In addition, the expression of of the QTLs interacting with environments was not consistent within each testing locality but varied between the years. This makes application of these QTL x E interactions difficult.

In this study, epistatic effects were detected for thousand grain weight and grading. The epistasis scan was repeated thirteen times, each with a 5% error threshold. It is unlikely, that both of these epistatic loci would have appeared by chance alone. In earlier barley

studies, epistasis or QTL x QTL interactions have been observed for example in QTLs for heading date (Laurie et al. 1995), yield (Thomas et al. 1995) and dormancy (Thomas et al. 1996). Epistatic interactions can be utilized by selecting for specific allele combinations instead of just pyramiding positive alleles (Zhu et al. 1999). However, QTL mapping experiments have provided only little evidence for epistasis compared to the strong classical evidence for the importance of epistasis especially in adapted germplasm of autogamous species (Paterson 1995, Allard 1988). This may at least partly result from insufficent replication and/or insufficient statistical resolution to detect interactions, in the presence of many QTLs with large main effects (Paterson 1995).

The breeders' aims concerning the Rolfi x Botnia cross were to combine the earliness and short straw of Rolfi with the larger grain and good malting quality of Botnia. Selecting for the 'early and short' Rolfi allele at the major main effect QTL on 2H and at the same time for the main effect Botnia alleles increasing the TGW on 5H and 6H could promote achieving the breeding goals across environments. Quality data collected from the same experiments as the agronomic data will be used to map QTLs affecting protein content and malting quality traits. Combined information on markers linked to important QTLs can be used to pyramid positive alleles for several traits. However, clustering of QTLs to the same chromosomal regions may render their application difficult if positive alleles are in repulsion phase. For breeding applications distinguishing between pleiotropic effects of a single gene and close linkage between independently acting genes is of major importance.

Results from each QTL mapping experiment apply, in principle, only to the mapping cross used for detecting the marker–QTL linkages. A low correspondence of marker–QTL associations was observed between the North American oat germplasm pool and a mapping cross (Beer et al. 1997). In addition, the effects of marker alleles were reversed in several cases, either due to epistatic or environmental effects, or due to recombinational erosion of the linkage disequilibrium. Still, marker associations with QTLs may be useful in a narrow-based germplasm where linkage disequilibrium has been retained between closely linked loci (Jannoo et al. 1999). It is probable that in the Finnish six-rowed barley germplasm, largely based on only seven ancestors, at least some ancestral marker-trait associations have remained in the modern varieties. Since QTL analysis of segregating progenies is very laborious, alternative approaches should also be considered for wider applicability of markers in breeding programs. Identification of genes controlling the quantitative traits and unravelling their DNA sequence would greatly enhance the use of markers in breeding for quantitative trait improvement. Different alleles of a QTL could be fingerprinted and variation in key regions of the genome followed in any germplasm. Although positional cloning demands great efforts, future developments in molecular biology techniques may increase the efficacy of genome analysis and make large scale sequence analysis of QTLs possible.

In many breeding programs narrow-based germplasm has been appended with resistance genes from landraces or wild species. Introgression is commonly done through a

backcrossing program, where the target gene or genes are transferred by repeated crossing to the adapted recipient and simultaneously the rest of the donor genome is eliminated. This procedure can be greatly enhanced using molecular markers (Young & Tanksley 1989). Using bulked segregant analysis, the *mlo*11 allele was tagged with RAPD markers to enhance breeding for powdery mildew resistance. Linkage disequilibrium between the closest marker and the *mlo*11 allele was maintained in the set of resistant and susceptible varieties tested. Still, a marker situated within the gene of interest could be more confidently used in any germplasm. Lately, the *Mlo* locus has been positionally cloned and the sequence of eleven mutation-induced recessive alleles (*mlo*) determined (Büschges et al. 1997). That work was the first occasion where chromosome landing (Tanksley et al. 1995) was used to isolate a gene in barley. Eight of the *mlo* alleles differ from the wild type allele with only one base substitution, but three include larger deletions (Büschges et al. 1997). Specific primers can be produced for alleles of interest and used in breeding programs: a marker located within the target gene offers an efficient tool for selection and backcross breeding.

Net blotch resistance represents a demanding challenge to barley breeders. Although several sources of resistance have been identified, environment, genetic background and the pathogen isolate have been reported to confer the expression of resistance genes (Afanasenko et al. 1999, Khan 1969). In order to map the resistance genes from CI9819, a linkage map of Rolfi x CI9819 was constructed. Map construction was much quicker than in the Rolfi x Botnia cross: parents differed three times more from each other and a more efficient marker system was used. We identified a major QTL for seedling resistance to net blotch on chromosome 6H. This gene is effective against the Finnish isolates of net blotch. In the near future we will test the resistance of the Rolfi x CI9819 doubled haploid mapping progeny to an array of net blotch isolates from different parts of the world. The effect of genetic background will be tested by backcrossing the resistance gene with the aid of markers to several different backgrounds. With the ‘near-isogenic lines’ for the resistance gene on 6H, differences in the cascade of defence reactions can be studied in detail. The dense marker cluster at the resistance gene can offer a starting point for positional cloning, although one has to bear in mind that the gene resides in the centromeric region, where recombination may be reduced by as much as ten fold compared with other regions of the chromosomes (Tanksley et al. 1992).

The resistance gene on 6H is located in the same region as a QTL for post-heading duration in the Rolfi x Botnia cross. A cluster of QTLs affecting yield, heading date and several malting quality traits has been recognized at the centromeric region of 6H in several barley crosses (Hayes et al. 1997). The putative linkage of this resistance gene to QTLs for important characters may hinder its use in breeding. The source of resistance used here is an Ethiopian barley line of poor agronomic type for Finnish conditions. To gain more information, we will test the field resistance of the doubled haploid progeny and at the same time observe the developmental rate of the lines. Fine mapping of the

centromeric region of 6H may help dissection of the effects of this region and offer tools for marker assisted selection.

Both the Rolfi x Botnia cross and the Rolfi x CI9819 cross segregate for quantitative field resistance to powdery mildew. An effort to map these genes will be made in the future. At the same time the linkage maps will be appended with candidate gene markers. These include clones from pathogenesis related genes, which will be used as RFLP probes, and resistance gene analogs, which are amplified by PCR using primers specific to sequences in common with plant disease resistance genes (Leister et al. 1996).

5. Conclusions

In Finland spring barley is cultivated at the northern species margin and the varieties have to be adapted to the short growing season demanding extremely rapid growth rhythm. In this study DNA-markers, including RAPDs, RFLPs, microsatellites and *BARE*-I retrotransposon markers, were used for assessing genetic diversity in Finnish six-rowed barley and for mapping and tagging genes affecting traits important in barley breeding.

Both pedigree information and analysis with RAPD markers indicate that Finnish six-rowed barley is genetically narrow-based. In this study, these two approaches gave uncorrelated measures of diversity. The level of diversity in RAPD markers has been maintained during variety breeding over the past 60 years.

Distorted segregation was apparent in both doubled haploid mapping progenies. In the Rolfi x Botnia cross skewed segregation was observed mainly on chromosomes 2H, 3H and 7H. Genes affecting anther culture response were located on chromosomes 2H, 3H and 4H. However, the observed segregation distortion could be explained by genes affecting anther culture for chromosome 3H only. Anther culture traits have not been mapped previously in barley.

QTLs for nine traits of agronomic importance were located on the narrow-based Rolfi x Botnia map. Several QTLs clustered at the same position, particularly on chromosomes 2H, 6H and 7H. QTL x environment interactions were detected for most of the traits. Loci affecting developmental rate before and after heading were partly different. Epistatic interactions for thousand grain weight and grading were noted. All straw length QTLs found in this study, as well as some of the QTLs affecting earliness and thousand grain weight, overlapped with previously found QTLs or mutations in corresponding traits. Loci for straw length and pre-flowering duration were mapped very precisely, the one-LOD support intervals being less than 3 cM. Information about QTLs can be utilized in mating design and selection to achieve an optimal combination of earliness, short straw and large grain. However, before using putative QTLs in breeding, the exact QTL locations and effects should be verified in independent lines of the mapping cross or in another genetic background.

Using a new marker type, REMAP, resistance genes for net blotch were mapped to chromosomes 6H and 5H in the Rolfi x CI9819 cross. The powdery mildew resistance locus, *Mlo*, was tagged with RAPD markers. Linked markers for net blotch and powdery mildew resistance may be used to speed up transfer of resistance from unadapted sources to highly adapted elite Finnish barley germplasm.

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