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“Mapping of new microsatellite markers and molecular identification of quantitative trait locus (QTL) for agronomically important traits in barley”

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Content

Abbreviations

A	adenine
AB	advanced backcross
AFLP	amplificated fragment length polymorphism
BC_1	first backcross generation
bp	basepair
C	cytosine
cDNA	complementary DNA
CIM	compound interval mapping
cM	centiMorgan
DAF	DNA amplification fingerprinting
DH	doubled haploid
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
et al.	et aleri
F_2	second generation after a cross
G	guanosine
GBMS	Gatersleben barley microsatellite
I/F	‘Igri’ × ‘Franka’
LOD	logarithm of odds
NIL	near isogenic line
PCR	polymerase chain reaction
R. r.	resistance
RIL	recombinant inbred line
QTL	quantitative trait locus
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
S/M	‘Steptoe’ × ‘Morex’
SDS	sodium dodeyl sulfate
SIM	simple interval mapping
SNP	single/simple nucleotide polymorphism
SSR	simple sequence repeat
STS	sequence tagged site
T	thymine
TE	Tris/EDTA
Tris	2-Amino-2 (hydroxymethyl)-1,3-propandiol

1 Summary

To enhance the marker density of existing genetic maps of barley (*Hordeum vulgare* L.), a new set of microsatellite markers containing dinucleotide motifs was developed from genomic clones. Out of 254 primer pairs tested, a total of 167 primer pairs were classified as functional in a panel of six barley cultivars and three *H. spontaneum* accessions and of those 127 primer pairs resulting in 133 loci were either mapped or located onto chromosomes. The polymorphism information content (PIC) ranged from 0.05 to 0.94 with an average of 0.60. The number of alleles per locus varied from 1 to 9. On average, 3.9 alleles per primer pair were observed. The RFLP frameworks of two previously published linkage maps were used to locate a total of 115 new microsatellite loci on at least one mapping population. The chromosomal assignment of 48 mapped loci was corroborated on a set of wheat-barley chromosome addition lines. Eighteen additional loci that were not polymorphic in the mapping populations were assigned to chromosomes by this method. The microsatellites were located on all seven linkage groups with four significant clusters in the centromeric regions of 2H, 3H, 6H and 7H. These newly developed microsatellites improve the density of existing barley microsatellite maps and can be used in genetic studies and breeding research.

Advanced backcross QTL (AB-QTL) analysis has been successfully applied in detecting and transferring quantitative trait loci (QTL) from unadapted germplasm into elite breeding lines for various plant species. With new and published microsatellites, QTLs for agronomic traits and malting quality parameters were localized in two AB-populations in spring barley.

A BC_3 doubled haploid population consisting of 181 lines derived from a German spring barley cultivar ‘Brenda’ as the recurrent parent and a wild species accession HS213 (*Hordeum spontaneum*) as the donor line was evaluated for yield and its components, and malting traits. A set of 60 microsatellite markers was used to genotype the population and phenotypic data were collected in two locations in Germany in continuous years. Altogether 42 significant QTLs were detected by single-marker regression and interval mapping. Most positive QTLs detected originated from the recurrent parent ‘Brenda’. However, a QTL, *hd2.2*, on chromosome 2HS from HS213 explained 19.3% and 22.4% of the phenotypic variation for yield and heading date, respectively. Consequently, the precise location of *hd2.2* was mapped into an interval of 6.5cM in a F_2 population consisting of 235 individuals developed from a

cross between a near isogenic line containing a defined donor segment at this locus and the variety ‘Brenda’. A total of 34 plants containing one single donor segment were found in the lines of the advanced backcross population, which can be regarded as defined introgression lines.

Eleven agronomic traits were characterized in a BC_3 population, containing 200 lines that originated from a cross of ‘Brenda’ as the recurrent parent and a wild species accession HS584 (*Hordeum spontaneum*) as donor parent. Seventeen nearly isogenic lines that contained a single donor segment were obtained by scanning with 107 polymorphic microsatellites distributed throughout the whole genome. A total of 81 QTLs were detected based on the combination of genotypes and phenotypic data gained from two locations in three years. Most positive effects for the trait yield were detected from the recurrent parent. However, for the heading date, of thirteen QTLs found in total, seven alleles from the wild species HS584 were identified to reduce the days to heading. Nine QTLs originating from ‘Brenda’ were confirmed in both populations, including *hd2.2* detected in the Brenda/HS213 population, which corresponded to *hd2.1* found in the ‘Brenda’/HS584 combination.

2 Zusammenfassung

Um die Markerdichte von existierenden genetischen Karten der Gerste (*Hordeum vulgare* L.) zu erhöhen, wurde ein neues Set von Mikrosatellitenmarkern mit Dinukleotidmotiven aus genomischen Klonen entwickelt. Von insgesamt 254 getesteten Primerpaaren, wurden 167 Primerpaare als funktional klassifiziert, nachdem sie auf sechs Gerstensorten und drei Akzessionen von *H. spontaneum* getestet worden waren. Davon konnten 127 Primerpaare, welche in 133 Loci resultierten, entweder kartiert oder auf Chromosomen lokalisiert werden. Der Informationsgehalt der Marker (‚polymorphism information content', kurz PIC) reichte von 0,05 bis 0,94 mit einem Durchschnitt von 0,60. Die Allelzahl per Locus variierte von 1 bis 9. Im Durchschnitt wurden 3,9 Allele pro Primerpaar beobachtet. Insgesamt 115 neue Mikrosatelltenloci wurden in die RFLP-Gerüste von zwei öffentlichen Kopplungskarten integriert. Die chromosomale Lokalisierung von 48 kartierten Loci wurde auf Weizen-Gerstenchromosomen-Additionslinien bestätigt und 18 weitere Loci, welche in den Kartierungspopulationen nicht polymorph waren, wurden auf diese Weise Chromosomen zugeordnet. Die Mikrosatelliten verteilten sich auf alle sieben Kopplungsgruppen mit signifikanten Anhäufungen in den Zentormer-nahen Regionen der Chromosomen 2H, 3H, 6H und 7H. Diese neu entwickelten Mikrosatelliten verbessern die Dichte der existierenden Gersten-Mikrosatelltenkarten und können für genetische und züchtungsrelevante Studien eingesetzt werden.

Markergestützte Rückkreuzung (‚Advanced backcross QTL analysis', kurz AB-QTL) ist erfolgreich zur Detektion und Übertragung von quantitativ vererbten Merkmalen (QTL) von nicht adaptiertem Pflanzenmaterial in Elite-Zuchtlinien bei verschiedenen Pflanzenarten eingesetzt worden. Mit Hilfe von neuen und zuvor publizierten Mikrosatellitenmarkern, wurden in dieser Studie QTLs für agronomisch wichtige Eigenschaften und Parameter für die Malzqualität in zwei markergestützten Rückkreuzungspopulationen von Sommergerste lokalisiert.

Eine BC_3-Doppelhaploidenpopulation aus 181 Linien, welche aus der Kreuzung zwischen der deutschen Sommergerstensorte ‚Brenda' als rekurrente Elter und der Wildart-Akzession HS213 (*Hordeum spontaneum*) als Donorlinie entwickelt entwickelt worden war, wurde für Ertrag und seine Komponenten sowie für Malzparameter evaluiert. Die Population wurde mit 60 Mikrosatellitenmarkern genotypisiert und phänotypische Daten wurden an zwei Standorten

in aufeinanderfolgenden Jahren erhoben. Es konnten insgesant 42 signifikante QTLs mit den Methoden der Einzelmarker-Regression und der Intervall-Kartierung gefunden werden. Die meisten positiven QTLs stammten dabei aus dem rekurrenten Elter ‚Brenda'. Ein QTL, *hd2.2*, auf Chromosom 2HS erklärte 19,3% bzw. 22,4% der phänotypischen Varianz für Ertrag und Zeitpunkt des Ährenschiebens. Daher wurde *hd.2.2* präzise als Einzelgen in ein Intervall von 6,1 cM in einer F_2-Population kartiert, welche aus einer Kreuzung zwischen einer nahezu iosgenen Linie mit definierten Donorfragment an diesem Locus und der Sorte ‚Brenda' entwickelt worden war. In der markergestützten Rückkreuzungspopulation wurden insgesamt 34 Linien mit einem spezifischen Donorsegment gefunden, welche als definierte Intergressionslinien betrachtet werden können.

Insgesamt elf agronomische Merkmale wurden in einer BC_3-Population bestehend aus 200 Linien, welche aus der Kreuzung von ‚Brenda' als rekurrenten Elter und der Wildart-Akzession HS584 (*Hordeum spontaneum*) als Donor-Elter resultierten, charakterisiert. Siebzehn nahezu isogene Linien mit einem einzigen Donorsegment wurden nach dem Testen mit 107 polymorphen Mikrosatelliten, welche gleichmäßig im Genom verteilt waren, gefunden. Insgesamt 81 QTLs wurden durch die Kombination von genotypischen und phänotypischen Daten von zwei Standorten in drei Jahren erhalten. Die meisten positiven Effekte für das Merkmal Ertrag stammten aus dem rekurrenten Elter. Von insgesamt 13 QTLs, welche für das Merkmal Zeitpunkt das Ährenschiebens gefunden wurden, stammten sieben Allele aus der Wildart HS584 , welche die Anzahl der Tage bis zum Ährenschieben reduzierten. Neun QTLs aus ‚Brenda' wurden in beiden Populationen bestätigt, einschließlich des QTL *hd2.2* in der Population ‚Brenda'/HS213, welcher mit *hd2.1* in der Kombination ‚Brenda'/HS584 korrespondierte.

3 Literature review

3.1 Yesterday and today of barley

Since the first description of the wild barley *Hordeum spontaneum* C. Koch in 1848, the opinions and disagreements about the common ancestor and the origin of cultivated barley (*H. vulgare*) arose more than one hundred years ago. Harlan and Zohary (1966) put forward an accepted view: Fertile Crescent as the unique center of origin of barley and *H. spontaneum* C. Koch as the ancestral parent of both two- and six-rowed ear types. However, with evidences found in Morocco (Molina-Cano and Conde. 1980; Salcedo *et al.* 1984; Molina-Cano *et al.* 1987, 1999, 2002; Moralejo *et al.* 1994;), and in southeastern Himalaya and Tibet (Ma *et al.* 1987; Xu 1982), the general agreement of the monocentric origin of wild barley was challenged. Therefore, the Near East, Tibet, Ethiopia, and the Western Mediterranean region were proposed as multi-centers of origin for barley.

Archaeological research proved that barley was one of the earliest cereals domesticated in the Near East, dating to 17,000 BC (Fischbeck 2002; Kislev *et al.* 1992). By the seventh millennium BC, domesticated barley had spread to western Anatolia and Iraq, and by the sixth millennium BC, had reached Greece (Zohary and Hopf 1988). Barley as a basic crop for the early irrigated agriculture emerged around the Nile Delta in ancient Egypt probably in the fifth millennium B.C. (Darby *et al.* 1977). Later, barley reached Spain and the Lower Rhine valley, respectively (Harlan 1995). By the third millennium B.C., cultivated barley entered Central and Northern Europe with further expansion (Körner-Grohne 1987). Egyptian hieroglyphic scripts suggested that barley was more important than wheat for human food because of its tolerance against salt, when the irrigated lands of southern Mesopotamia began to salt up (Jacobasen and Adams 1958). By the mid-18th century, Spaniards introduced barley quickly to their colonies from California to Chile (Harlan 1995). The cultivated varieties of barley in Oceania originated from immigrants from the British Empire (Fischbeck 2002). Conscious selection of desired genotypes by farmers at an early stage, together with natural selection, increased the diversity and created the rich genepool – the source of variation found today in local varieties. These landraces also formed the basic material for modern plant breeding, which started about 150 years ago (von Bothmer *et al.* 2003).

With the development of malting and brewing industries, barley became the major source of raw material. Barley is favored by temperate conditions of climate, and some of the best

malting grain is consequently produced in districts bordering the sea coast, and in more or less isolated areas similarly favored by equable weather conditions (Hunter 1951). Meanwhile, barley production has increased significantly as the demand for livestock feed had grown. Therefore, barley is a special-purpose grain rather than a general market crop, finding its greatest use as a substitute for maize in animal feeding and for malting. Consequently, from the middle of the twentieth century, barley occupied the fourth position in the world's cereal acreage, following the larger acreages of wheat, rice, and maize, each of which covers more than 20% of total production acreage (see Table 3.1, FAO 2002).

Fischbeck (2002, 2003) estimated that nearly 85 % of the current world barley production was used for feeding animals, and most of the rest for malting industry. Consequently, barley was transformed into the human food supply system, indirectly. In the area of EU, feed domestic consumption rate was 70% in 2002 (USDA, 2003). Moreover, the need for malting barley as a major material for the brewing industry should be taken in consideration while the total world beer production increases steadily. Europeans produce 25% of the total world beer production, which is equal to 320 million hectoliters of beer each year (The Brewers of Europe). The famous German beer industries should be mentioned with its over thousand brewing plants owning 100 million hectoliters production ability, leading to the importance of malting barley in German cereal production. For these reasons, the focus of breeding is not increasing the yield of barley production but improvement of the malting barley quality.

Table 3.1 World cereals acreage (million ha)

Year	Wheat		Rice		Maize		Barley		Sorghum	Millet	Oat	Rye	Triticale	Cereals Total
	Acre.	%	Acre.	%	Acre.	%	Acre.	%	Acre.	Acre.	Acre.	Acre.	Acre.	Acre.
1962	207.6	31.74	119.6	18.29	103.4	15.81	**58.0**	**8.86**	46.6	43.0	33.8	30.3	-	653.9
1967	219.7	32.32	127.7	18.78	112.3	16.52	**60.9**	**8.95**	50.5	45.3	29.8	24.0	-	680.0
1972	213.8	31.55	132.4	19.54	114.9	16.96	**72.9**	**10.77**	44.5	41.4	29.3	18.2	0.43	677.5
1977	228.6	31.78	143.9	20.00	125.2	17.41	**82.0**	**11.40**	47.3	40.3	29.0	14.8	0.51	719.3
1982	238.5	33.38	141.8	19.85	124.4	17.40	**77.9**	**10.90**	45.6	35.6	25.6	17.3	0.69	714.6
1987	220.6	31.62	141.3	20.26	129.9	18.63	**78.1**	**11.19**	46.0	34.0	23.0	16.3	1.27	697.5
1992	222.4	31.37	147.3	20.77	137.0	19.32	**73.5**	**10.37**	46.3	37.5	19.5	14.7	2.43	709.1
1997	226.3	32.34	151.0	21.58	141.3	20.19	**63.4**	**9.07**	45.1	36.2	15.5	10.8	2.60	699.7
2002	210.6	32.00	147.1	22.35	138.8	21.08	**52.2**	**7.92**	42.6	33.4	13.5	9.5	3.07	658.2

Data source: FAO 2003

3.2 Molecular mapping of the barley genome

3.2.1 Genetic mapping in barley

Barley (*Hordeum vulgare* L.) is not only an important crop worldwide but also an excellent system for genome mapping and map-based analyses (Costa *et al.* 2001), because its chromosomes are homoeologous to cultivated wheat and rye, respectively (Hori *et al.* 2003). The nuclear DNA content often varies somewhat among different cultivars (Bennett 1985). The nuclear genome size of barley (*Hordeum vulgare* L.) is approximately 4.9×10^9bp/1C (Arumuganathan and Earle 1991), a bit smaller than 5.3×10^9bp/1C originally reported by Bennett and Smith (1976). The cytology and genetics of barley was reviewed by Smith (1951). It is a diploid ($2n = 2x = 14$), self-pollinated species. Seven barley chromosomes were identified and labeled based on their sizes and characteristics (Burnham and Hagberg 1956). Chromosomes 1 through 5 differ in their sizes measured at mitotic metaphase, with chromosome 1 being the longest and chromosome 5 being the shortest; chromosomes 6 and 7 have satellites, with chromosome 6 having the larger satellite and chromosome 7 having the smaller satellite (Kleinhofs and Han 2002). Since the barley chromosomes have the same DNA content as those in other members of the Triticeae, and the gene loci in barley are largely collinear with the loci in other members of the Triticeae, with few ancestral translocations involving whole chromosome segments, chromosomes 1 to 7 of barley (*Hordeum vulgare* L.) were redesignated as chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H respectively (Singh and Tsuchiya 1982; Linde-Laursen 1997). The barley genome present in the variety ‘Betzes’ became the reference genome in the barley to which definitions of translocations, short arm/long arm reversals, etc. were standard in all species. Meanwhile, wheat barley chromosome addition lines were available for ‘Betzes’, so other Triticeae workers have an incentive to test their probes on barley (Islam *et al.* 1981).

Construction of a linkage map with molecular markers is a key step in the linkage analysis of biologically or agronomically important traits. Dense genetic maps of cereals contribute substantially to the positional cloning of important genes and provide a tool for evolutionary studies, as well as the characterization of germplasm and gene discovery. Following the rediscovery of Mendel’s laws of heredity, the first linkage maps for seven barley chromosomes were published by Robertson (1939). Smith (1951) gave seven linkage groups in his review based on the summaries of linkage studies (Robertson *et al.* 1941, 1947). Of totally 103 genes were listed, which represented morphological, cytological, and biochemical

characters. Cytogenetic methods such as translocation analysis and the primary trisomic method were introduced in the early 1950s and greatly contributed to the establishment of cytogenetic linkage maps (Tsuchiya 1984). More than 60 isozyme markers were detected in barley (Brown *et al.* 1978, 1989; Nielsen and Johansen 1986) and a well-developed classical genomic map was constructed for barley using isozyme and morphological markers (Sogaard and von-Wettstein-Knowles 1987).

3.2.2 Molecular markers

3.2.2.1 RFLP-markers

The development of restriction fragment length polymorphism (RFLP) for high density genomic mapping in human (Botstein *et al.* 1980) provided a new technique which overcame some of the problems associated with isozymes and proteins. Since then, RFLP markers have been widely used to construct linkage maps for several crop species, including maize (Helentjaris *et al.* 1986), rice (McCouch *et al.* 1988), and tomato (Tanksley *et al.* 1992). Restriction endonucleases are enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Since genomic DNA differs in nucleotide sequences, fragments of different sizes may be generated for different plant accessions when digested with restriction endonucleases and separated by gel electrophoresis. The fragmented DNA can be transferred from agarose gels to Nylon filters by Southern blotting. By hybridization with cDNA probes or other cloned single- or low-copy DNA elements labeled radioactively, fragments of different sizes will be observed on Nylon filters containing digested DNA by autoradiography. Polymorphic cDNA probes and other cloned single- or low-copy DNA elements are called RFLP-markers.

To date, extended linkage maps based on RFLP markers have been constructed for a great number of agronomic crops and other plant species. The first application of RFLP genetic mapping in barley was on chromosome 6H by Kleinhofs *et al.* (1988). Later, the first genetic map using RFLP as molecular markers in barley emerged (Shin *et al.* 1990). Consequently, more detailed and high-density RFLP maps were published (Graner *et al.* 1991; Heun *et al.* 1991; Kleinhofs *et al.* 1993). In the following years, although RFLP analysis requires large quantities of high quality DNA, and detection of RFLPs by Southern blot hybridization is laborious and time-consuming, which make this assay undesirable for plant-breeding projects with high-throughout requirements, this technique was a powerful tool for barley in comparative mapping studies among Gramineae species (Devos and Gale 1993; Sherman *et*

al. 1995), the construction of a consensus map in barley (Langridge *et al.* 1995), and gene mapping (Hinze *et al.* 1991; Laurie *et al.* 1995).

3.2.2.2 RAPD-markers

PCR (Polymerase Chain Reaction) has revolutionized molecular genetics. The development of a PCR-based arbitrarily primed genetic assay called RAPD (Random Amplified Polymorphic DNA, Williams *et al.* 1990), AP-PCR (Arbitrarily Primed PCR, Caetano-Anollés *et al.* 1991) or DAF (DNA Amplification Fingerprinting, Welsh and McClelland 1990) has been widely used for the construction of genetic maps (Bowditch *et al.* 1993; Kresovich *et al.* 1992; Reiter *et al.* 1992; Williams *et al.* 1993a, 1993b) and has greatly changed the prospects for application of molecular markers to study populations and to accelerate breeding (Rafalski *et al.* 1991; Rafalski and Tingey 1993). In particular, RAPD markers provide a very powerful tool to generate relatively dense linkage maps in a short period of time. Amplification products of the RAPD assay are specific DNA fragments with arbitrarily 10-base oligonucleotides as primer. Polymorphisms detected between individuals presumably result from numerous changes including sequence differences in one or both of the primer binding sites, insertion/deletion events or rearrangement in priming sites or in the internal amplified sequence. They are defined by the presence or absence of a particular amplified product (Welsh *et al.* 1992; Williams *et al.* 1990). Thefeore, the arbitrarily primed PCR products are usually dominant markers and cannot distinguish homozygous and heterozygous states.

Compared with the RFLPs, the advantage of the arbitrarily primed PCR techniques such as RAPDs were, the requirement of small amounts of DNA (5-20ng), the rapidity to screen for polymorphisms, the efficiency to generate a large number of markers for genomic mapping and the potential automation of the technique (Nelson *et al.* 1992). Since the first two reports about detection and mapping of the RAPD markers in barley by Dawson *et al.* (1993) and Giese *et al.* (1994), RAPD analysis as a simple and easy-to-handle method was used for tagging of genes, such as genes for resistance to barley blotch (Kutcher *et al.* 1996; Molnar *et al.* 2000) and barley yellow dwarf virus (Wang and Zhang 1996; Zhang *et al.* 2001).

3.2.2.3 AFLP-markers

Amplified Fragment Length Polymorphism (AFLP) as a PCR-based fingerprinting technique was first described by Zebeau and Vos (1993). The AFLP^{TM} technology is under patent

owned by KeyGene N.V. (www.keygene.com). It is based on the selective amplification of a subset of genomic restriction fragments using PCR (Vos *et al.* 1995). Genomic DNA is digested with restriction endonucleases and ligated to synthetic adaptors. Thus, the sequence of the adapters and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments by PCR. Selective nucleotides extending into the restriction fragments are added to the 3' ends of the PCR primers such that only a subset of the restriction fragments are recognized. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified. The subset of amplified fragments is then analyzed by denaturing polyacrylamide gel electrophoresis to generate the fingerprint. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. The level of polymorphism is species specific. Compared with allozymes, RAPDs, and RFLPs, AFLPs have superior performance in the time and cost efficiency, replicability and resolution, except that the AFLP method primarily generates dominant rather than co-dominant markers (Mueller and Wolfenbarger 1999).

Since the first report about AFLP mapping in barley was published (Becker *et al.* 1995), several maps of AFLP makers have been constructed (Qi and Lindhout 1997; Qi *et al.* 1998; Mano *et al.* 2001; Hori *et al.* 2003) Later, it became an important tool in barley genetic research, including the investigation of the origin of barley (Allaby and Brown 2003; Badr *et al.* 2000), diversity studies (Ellis *et al.* 1997; Waugh *et al.* 1997; Russell *et al.* 1997b), mapping of QTL (Pakniyat *et al.* 1997; Powell *et al.* 1997b; Dahleen *et al.* 2003), detection of disease resistance genes (Toojinda *et al.* 1998), and fine mapping of disease resistance genes such as *mlo* (Simons *et al.* 1997), *Mla* (Schwarz *et al.* 1999) and *Rph15* (Weerasena *et al.* 2003).

3.2.2.4 STS-markers

An STS (Sequence Tagged Sites, Olson *et al.* 1989) is a unique, single copy segment of the genome whose DNA sequence is known by sequencing and which can be amplified by specific PCR. With a set of primers of about 20-25 nucleotides in length derived from a stretch of DNA with a known sequence, unique DNA segments of about 90-300 bps can be amplified. A combination of advantages (markers are PCR based, no clone maintenance or distribution is necessary) and their co-dominant mode of inheritance make STS markers an

important marker system in crop plants (Williams *et al.* 1991; Melz *et al.* 1992; Konieczny and Ausubel 1993). The main advantage of STS markers lies in the speed with which they can be analyzed once PCR primer pairs have been identified.

Following the first reported mapping of STS markers in barley (Tragoonrung *et al.* 1992), a large amount of RFLP markers was converted to STSs for cultivar fingerprinting purpose (Faccioli *et al.* 1995), for physical mapping (Korzun and Künzel 1997) and for mapping specific genes (Blake *et al.* 1996; Graner *et al.* 1996; Graner and Tekauz 1996; Larson *et al.* 1996; Mohler and Jahoor 1996). Later, a genetic map covered the entire barley genome (Mano *et al.* 1999) and new sources for development of STS maker in barley were available from EST sequencing results (Michalek *et al.* 1999).

3.2.2.5 SSR-markers

Simple sequence repeats (SSRs) (Tautz 1989), also called microsatellites, are stretches of DNA consisting of tandemly repeated short units of 1-6 basepairs in length, and are co-dominantly inherited (Johansson *et al.* 1992). Such motifs are abundant and highly polymorphic in the genome of eukaryotes (Tóth *et al.* 2000). Microsatellites can be found anywhere in the genome, both in protein-coding and noncoding regions. The conserved sequences in the flanking regions of simple sequence repeats can be designed as a pair of specific primers to detect the DNA length polymorphism via the polymerase chain reaction (Litt and Luty 1989; Weber and May 1989). A high level of polymorphism is to be expected because of the proposed mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz *et al.* 1986). The SSR markers can be identified by sequencing microsatellite-containing clones isolated from small-insert genomic DNA libraries via hybridization with synthetic oligonucleotide probes, a method which is time-consuming and relatively expensive. A low cost way of SSRs development is screening of sequences in the public database.

The most frequently found repetitive motifs of mono-, di-, tri-, or tetranucleotide units are $(A)_n$, $(GA)_n$, $(TAT)_n$ and $(GATA)_n$ in plants (De Vienne *et al.* 2003). The most abundant dimeric microsatellite in several well-known mammals is the AC repeat (Beckmann and Weber 1992), while in many plant species they are AT or GA repeat (Wang *et al.* 1994). More than 75% of the barley genome comprises repetitive DNA sequences (Flavell *et al.* 1977). It is estimated that the barley genome contains one GA repeat every 330kb and one GT

repeat every 620kb (Liu *et al.* 1996b), which is in agreement to the findings that GA repeats occur in barley at a higher frequency than GT repeats by Struss and Plieske (1998). Similar results were obtained with other important crops, such as wheat (Plaschke *et al.* 1995; Röder *et al.* 1995), rice (Wu and Tanksley 1993), and maize (Gupta and Varshney 2000). Among trinucleotide repeats in barley, $(CCG)_n$, $(AGG)_n$ and $(AGC)_n$ repeats are the most-frequent motifs while $(ACGT)_n$ and $(ACAT)_n$ in tetrameric microsatellites (Thiel *et al.* 2003).

The discovery of microsatellites has significantly increased the marker density of linkage maps for some mammals, human (Engelstein *et al.* 1993; Dib *et al.* 1996) and mouse (Dietrich *et al.* 1996). Molecular linkage maps in many model plants and crops were improved rapidly by the addition of SSR markers, such as in *Arabidopsis* (Bell and Ecker 1994), rice (McCouch *et al.* 1997), wheat (Röder *et al.* 1998) and maize (Senior and Heun 1993). The informative value of microsatellite markers for genetic studies and as a powerful tool for barley breeding was confirmed in several studies (Maroof *et al.* 1994; Becker and Heun 1995; Liu *et al.* 1996b; Struss and Plieske 1998). Among several important DNA marker systems, SSR markers showed the highest polymorphism, followed by RFLPs, RAPDs and AFLPs (Russell *et al.* 1997b). A second-generation linkage map of barley using only PCR-based microsatellite markers was constructed (Ramsay *et al.* 2000). Besides microsatellites derived from genomic clones, also ESTs were exploited for the development of PCR-based SSR-markers (Thiel *et al.* 2003; Pillen *et al.* 2000; Holton *et al.* 2002).

3.2.2.6 SNP-markers

The most general type of polymorphism, known as single nucleotide polymorphism (SNP), results from a single base mutation which substitutes one base for another. Other types of genetic polymorphisms result from the insertion or deletion of a section of DNA, which include microsatellite repeat sequences and gross genetic losses and rearrangements. Polymorphisms can be caused by mutations ranging from a single nucleotide base change to variations in several hundred bases. The mining of SNPs involving non gel-based assays and has recently been facilitated by the availability of genome-wide sequences and EST databases. A genetic map of the human genome was constructed showing the location of 2227 SNPs (Wang *et al.* 1998). Scientists have identified about 1.4 million locations where single-base DNA differences (SNPs) occur in humans. The polymorphisms of SNP loci also were characterized in many other plants, such as rice (Ayres *et al.* 1997), beet (Scheider *et al.* 2001), maize (Tenaillon *et al.* 2001), and soybean (Zhu *et al.* 2003). Many SNPs have been

found with screening entire genome sequences in *Arabidopis thaliana* (The Arabidopsis Genome Initiative 2000) and *Oryza sativa* (Goff *et al.* 2002; Yu *et al*, 2002). For many larger genomes, SNPs can be detected with scanning their EST sequences. For barley, SNPs have been successfully developed and applied in genetics studies (Kota *et al.* 2001, 2003; Kanazin *et al.* 2002; Russell *et al.* 2004; Thiel *et al.* 2004). Due to their abundance and slow mutation rate within generations, they are thought to be the next generation of genetic markers that can be used in a myriad of important biological, genetic, pharmacological, and medical applications. A high-resolution map of barley will be published in the near future, containing 1,044 loci and including 611 RFLP loci, 190 SSR loci and 255 SNP loci (Prasad et al. personal communication).

3.3 Mapping of quantitative trait loci (QTL)

3.3.1 Mapping and analysis of quantitative trait loci (QTL)

Traditional genetic studies have concentrated on dichotomous traits such as the presence or absence of a disease resistance in plant. Such traits are often the result of a mutation at a single gene. However, most of the agronomically important traits exhibit a continuous range of phenotypic variation, which is more or less normally distributed (Kearsey and Farquhar 1998) and can be explained by the independent action and potential interaction of many discrete genes and are affected by environmental factors (Kleinhofs and Han 2002). The precise number of genes involved is usually not known (Mather 1949). A major gene affecting a quantitative trait that has been localized to a chromosome is called a quantitative trait locus (QTL) (Gelderman 1975). Before 1980, the classical quantitative genetics was mainly based on statistical techniques, such as means, variances and covariances of relatives, with no knowledge of the number and location of the genes that underlie them (van-Rijn 2001). The first report of an association between a morphological marker locus and a quantitative trait was reported by Sax (1923), between a pigment locus and seed size in the bean, *Phaselous vulgaris*, demonstrating that the variation of size differences of the seed-coat followed the fundamental Mendelian properties of segregation and recombination. A key development in the field of complex trait analysis was the establishment of large collections of molecular and genetic markers, which offered the possibility of mapping QTLs depending on the level of resolution and density of the genetic maps. Recent and continuing advances in molecular genetics and statistical techniques make it possible to identify the chromosomal regions where these QTL are located (Tanksley 1993).

The statistical analyses of associations between phenotype and genotype in a population to detect quantitative trait loci include single-marker mapping (Edwards *et al.* 1987; Luo and Kearsey 1989), interval mapping (Lander and Botstein 1989), and composite interval mapping (CIM) (Zeng 1993; Zeng 1994), plus multiple trait mapping (Jiang and Zeng 1995; Ronin *et al.* 1995).

3.3.1.1 Single-marker tests

The simplest method for QTL mapping is single-marker mapping, including *t*-test, and analysis of variance (ANOVA) and simple linear regression, which assess the segregation of a phenotype with respect to a marker genotype (Soller 1976), according to these principles:

classify progeny by marker genotype, and compare phenotypic mean between classes (*t*-test or ANOVA); A significant difference indicates that a marker is linked to a QTL. The difference between the phenotypic means provides an estimate of the QTL effect. This approach can indicate which markers linked to potential QTLs are significantly associated with the quantitative trait investigated. In short, QTL location is indicated only by looking at which markers give the greatest differences between genotype group averages. Depending on the density of markers, the apparent QTL effect at a given marker may be smaller than the true QTL effect as a result of recombination between the marker and the QTL. The advantage of this method is a simple procedure that can be accomplished by a standard statistical analysis software package, such as SAS and Minitab. In contrast, the main weakness of single-marker tests is the failure to provide an accurate estimate of QTL location or recombination frequency between the marker and the QTL, because the evaluation of individual markers is independently, and without reference to their position or order (Doerge 2002).

3.3.1.2 Single interval mapping (SIM)

Interval mapping is probably the most familiar method of QTL analysis. The introduction of interval mapping offered a new strategy to discern weak effects from genetic distance between marker locus and putative QTL using the power of a complete genetic map. The interval that are defined by ordered pairs of markers are searched in increments, and statistical methods are used to test whether a QTL is likely to be present at the location within the intervals or not. The principle behind interval mapping is to test a model for the presence of a QTL at many positions between two mapped marker loci. The model is fit, and its goodness is tested using the method of maximum likelihood. If it is assumed that a QTL is located between two markers, the 2-locus marker genotypes contain mixtures of QTL genotypes each. Maximum likelihood involves searching for QTL parameters that give the best approximation for quantitative trait distributions that are observed for each marker class. Models are evaluated by computing the likelihood of the observed distributions with and without fitting a QTL effect. The LOD (logarithm of the odds) score is the log of the ratio between the null hypothesis (no QTL) and the alternative hypothesis (QTL at the testing position). Large LOD scores correspond to greater evidence for the presence of a QTL. The best estimate of the location of the QTLs is given by the chromosomal location that corresponds to the highest significant likelihood ratio. The LOD score is calculated at each position of the genome. In the case of many missing genotypes and large gaps on the map, the missing data are replaced

by probabilities estimated from the nearest flanking markers (Broman 2001). Until now, many software packages based on interval mapping were developed for QTL mapping, such as MAPMAKER/QTL (Lincoln *et al.* 1992) and QGene (Nelson 1997). In comparison to single-marker mapping, the benefits of these programs are a curve available across the genetic map, indicating the evidence of QTL location and which allows the inference of QTLs to positions or gaps between two markers in order to make proper analysis for incomplete marker genotype data. Meanwhile, analysis can be used for testing the presence of genotyping errors (Lincoln and Lander 1992).

3.3.1.3 Composite interval mapping (CIM)

There are two problems with single interval mapping (SIM) method as a result from single QTL model mentioned above. One is that the effects of additional QTL will contribute to sampling variance. The other is that combined effects of two linked QTLs will cause biased estimates. The ideal solution would be to fit a model that contains the effects of all QTL. However, the tremendous number of potential QTL and their interactions will lead to innumerable statistical models and heavy computational demands as using statistical approaches to locate multiple QTL. To deal with the this problem, several key papers were published (Jansen 1993; Jansen and Stam 1994; Zeng 1993; Zeng 1994). The approach of composite interval mapping assesses the probability that an interval between two markers is associated with a QTL that affects the trait of interest, and is as well controlling for the effects of other background markers on the trait. In theory, CIM gives more power and precision than SIM because the effects of other QTL are not present as residual variance. Furthermore, CIM can remove the bias that would normally be caused by QTL that are linked to the position being tested. The key problem with CIM concerns the choice of suitable background markers to serve as covariates.

3.3.2 QTLs in barley

3.3.2.1 Mapping of agronomic traits

For over a decade, with development of molecular approaches, QTL analysis was used to detect yield and fecundity-related traits. The most important agronomic trait in barley is yield, which is very complex. Many QTLs affecting yield were mapped on seven chromosomes throughout the whole genome. As summarized in Table 3.2, different numbers of QTL for yield were detected in different populations and environmental conditions. However, many of them are very difficult to be validated. A six-row cross, Steptoe × Morex (SM), has been

extensively developed as mapping population. Based on the phenotypic data from sixteen locations, 14 QTLs for yield were mapped on seven chromosomes in this mapping population (Hayes *et al.* 1993). Of them, only five on 2H, 3H, 5H, and 6H were confirmed in the same cross by Zhu *et al.* (1999a), Romagosa *et al.* (1996 and 1999b) and Han *et al.* (1999), respectively. The other two agronomic traits, heading date and plant height were easier to be investigated and were evaluated as additional important information to total yield.

Table 3.2 Major agronomic traits identified by QTL analyses in barley

Agronomic trait	Chromosome	Reference
Yield	1H, 2H, 3H, 4H, 5H, 6H, 7H	Hayes *et al.* 1993; Backes *et al.* 1995; CanteroMartinez *et al.* 1995; Thomas *et al.* 1995; Kjaer and Jensen 1996; Romagosa *et al.* 1996 and 1999b; Tinker *et al.* 1996; Bezant *et al.* 1997a; Powell *et al.* 1997a; Voltas *et al.* 1999a; Yin *et al.* 1999; Zhu *et al.* 1999a; Kandemir *et al.* 2000a; Marquez-Cedillo *et al.* 2001; Ellis *et al.* 2002 ; Pillen *et al.* 2003
Grain nmber per ear	1H, 2H, 3H, 4H, 5H, 6H	Bezant *et al.* 1997a; Pillen *et al.* 2003
Grain weight per ear	1H, 2H, 3H, 4H, 5H, 6H, 7H	Bezant *et al.* 1997a; Voltas *et al.* 1999b; 1999c
Grain weight per plant	1H, 2H, 3H, 4H, 5H, 6H, 7H	Bezant *et al.* 1997a
Thousand grain weight	2H, 3H, 4H, 5H, 6H, 7H	Thomas *et al.* 1995; Kjaer and Jensen 1996; Bezant *et al.* 1997a; Powell *et al.* 1997a; Hori *et al.* 2003; Pillen *et al.* 2003
Ear breaking	2H, 4H, 6H	Backes *et al.* 1995
Ear length	2H	Kjaer and Jensen 1996
Spike number	3H	Kandemir *et al.* 2000a; 2000b
Root length	5H	Jefferies *et al.* 1999
Plant height	1H, 2H, 3H, 4H, 5H, 6H, 7H	Hayes *et al.* 1993; Thomas *et al.* 1995; Bezant *et al.* 1996; Tinker *et al.* 1996; Powell *et al.* 1997b; Qi *et al.* 1998; De la Pena *et al.* 1999; Borem *et al.* 1999; Yin *et al.* 1999; Zhu *et al.* 1999b; Marquez-Cedillo *et al.* 2001; Teulat *et al.* 2001; Hori *et al.* 2003; Pillen *et al.* 2003
Heading date	1H, 2H, 3H, 4H, 5H, 6H, 7H	Hackett *et al.* 1992; Hayes *et al.* 1993; Backes *et al.* 1995; Laurie *et al.* 1995; Thomas *et al.* 1995; Bezant *et al.* 1996; Tinker *et al.* 1996; Powell *et al.* 1997b; Qi *et al.* 1998; De la Pena *et al.* 1999; Zhu *et al.* 1999b; Borem *et al.* 1999; Yin *et al.* 1999; Marquez-Cedillo *et al.* 2001; Pillen *et al.* 2003
Kernel weight	1H, 2H, 4H, 5H	Backes *et al.* 1995; Mather *et al.* 1997
Kernel length	4H, 7H	Backes *et al.* 1995; Mather *et al.* 1997 ; Marquez-Cedillo *et al.* 2000;
Kernel plumpness	1H, 2H, 4H, 5H, 7H	Mather *et al.* 1997; Marquez-Cedillo *et al.* 2000
Kernel shape	1H	Backes *et al.* 1995
Dwarfing	2H, 3H, 4H	Franckowiak 1997; Börner *et al.* 1999; Ivandic *et al.* 1999; Ellis *et al.* 2002

3.3.2.2 Mapping of malting quality

The improvement of malting quality also is an important objective for barley breeders because of its major industry usage in brewing. However, malting quality is a complex character related to many traits, such as malt extract percentage, total grain protein, soluble protein, ratio of soluble/total protein, β–glucan content, kernel plumpness, α-amylase activity,

diastatic power, and malt β-glucanase (Burger and LaBerge 1985). The malting process involves the interactions of a number of genes expressed during seed germination and development, depending on the temperature required during the reaction process (Gu *et al.* 2001). Not only yield and its components are affected by genetic factors and environments, malting qualities of barley are also influenced by these factors. The enzymes α-amylase and β-amylase convert gelatinized starch and glucans into sugars (Henson and Stone 1988; Ko and Henry 1994). Five QTLs for malting quality were detected around the amylase loci on 2H, 4H and 6H (Hayes *et al.* 1993), which was the first report for QTL analysis for malting quality. Han *et al.* (1995) mapped 12 loci for β-glucan content in barley grain and activity of β-glucanase that degrades cell wall β-glucan in malting process, respectively. Summarizing many data over years and locations, a region on chromosome 7H near the centromere was identified to be a complex QTL region controlling malt extract, α-amylase activity, diastatic power and β-glucanase, etc. (Hayes *et al.* 2003). Higher protein content and ratio of soluble/total protein affect the quality of products and increase the cost of production. Though storage and structural protein (*GluA*, *GluB* and *Glu.c1*) were mapped on 1H (Kleinhofs *et al.* 1993), QTLs for protein content and ratio were mapped on seven chromosomes (Oziel *et al.* 1996; Larson *et al.* 1997; Mather *et al.* 1997 and Marquez-Cedillo *et al.* 2000). Moreover, a large number of QTL for seed dormancy and germination were reported by Han *et al.* (1996 and 1999), Thomas *et al.* (1996), Romagosa *et al.* (1999a) and Gao *et al.* (2003) (see Table 3.3). Of them, a major dormancy locus on chromosome 5H was verified in different labs.

Table 3.3 Main traits for malting quality in barley

Malting trait	Chromosome	Reference
α-amylase	1H, 2H, 4H, 5H, 6H, 7H	Hayes et al. 1993; Ko and Henry 1994; Weining et al. 1994; Ko et al. 1996; Oziel et al. 1996; Han et al. 1997; Ullrich et al. 1997; Marquez-Cedillo et al. 2000
β-amylase	2H, 4H	Eglinton et al. 1998; Erkkila et al. 1998; Hayes et al. 1993
β-glucan	1H, 3H, 4H, 5H, 7H	Han et al. 1995, 1997; Mather et al. 1997; Ullrich et al. 1997
α-glucosidase	7H	Konishi et al. 1994; Im and Henson 1995
β-glucanase	1H, 2H, 4H, 5H, 7H	Loi et al. 1988; Macleod et al. 1991; Vietor et al. 1993; Han et al. 1995; Li et al. 1999
Diastatic Power	1H, 2H, 3H, 4H, 5H, 6H, 7H	Hayes et al. 1993; Oziel et al. 1996; Thomas et al. 1996; Han et al. 1997; Larson et al. 1997; Mather et al. 1997; Powell et al. 1997b; Ullrich et al. 1997; Marquez-Cedillo et al. 2000
Fermentability	2H, 3H, 5H	Swanston et al. 1999
Grain nitrogen	1H, 2H, 3H, 4H, 5H, 6H, 7H	Bezant et al. 1997a, b
Grain Protein	1H, 2H, 3H, 4H, 5H, 6H, 7H	Hayes et al. 1993; Oziel et al. 1996 ; Larson et al. 1997; Mather et al. 1997; Marquez-Cedillo et al. 2000
Sol./tot.protein	1H, 2H, 3H, 4H, 5H	Oziel et al. 1996 ; Mather et al. 1997; Marquez-Cedillo et al. 2000
Hot water extract	1H, 2H, 3H, 4H, 5H, 6H	Thomas et al. 1996 ; Bezant et al. 1997b; Powell et al. 1997b

Malting trait	Chromosome	Reference
Malt extract	1H, 2H, 4H, 5H, 6H, 7H	Hayes et al. 1993; Oziel et al. 1996; Ullrich et al. 1997; Marquez-Cedillo et al. 2000
Seed dormancy	5H	Han et al. 1996, 1999; Romagosa et al. 1999a; Gao et al. 2003
Germination	2H, 3H, 5H, 6H, 7H	Thomas et al. 1996; Mano and Takeda 1997a, b; Powell et al. 1997a

3.3.2.3 Mapping disease resistance gene

The conclusion that on average yield losses of 10.5% in barley are caused by diseases is based on 15,700 literature references and 3,700 field trials (Oerke and Dehne 1997). Jørgensen (1988) published a list of 83 loci rendering resistances to important barley diseases. Graner (1996b) provided a valuable review of molecular mapping of qualitative and quantitative disease resistance genes. Current state of resistance study and breeding in barley were summarized in detail by Kleinhofs and Han (2002), Chelkowski *et al.* (2003) and Weibull *et al.* (2003). Barley growth is mainly damaged by fungal diseases and virus diseases (see Table 3.4). Fungal diseases comprise powdery mildew, scald, rust diseases (leaf rust, stripe rust and stem rust), net blotch and others. Barley is attacked by several viruses, which are the barley yellow dwarf viruses, the cereal yellow dwarf virus, the barley stripe mosaic virus, the barley yellow streak mosaic virus, and the wheat dwarf virus.

Table 3.4 Major resistance genes against 15 fungal pathogens, four virus and two pests in barley (Chelkowski *et al.* 2003; Weibull *et al.* 2003)

Gene symbol	Pathogen and pest caused disease	No. of genes
Rph	*Puccinia hordi* (leaf rust)	17
Rpg	*Puccinia graminis*(stem rust)	4
Rps	*Puccinia striiformis* f. sp. *hordi*	4
Ml (Mlo, Mla, MILa and Reg)	*Erysiphe graminis* f. sp. *hordei* (powdery mildew)	23
Rcs	*Cochliobolus sativus* (spot blotch)	5
Rpt	*Pyrenophora teres* (net blotch)	6
Rdg (Rhg)	*Pyrenophora graminea* (barley stripe)	3
Rrs (Rh)	*Rhynchosporium secalis* (scald)	14
Run (un)	*Ustilago nuda* (loose smut)	8
Ung	*Ustilago nigra* (semiloose smut)	1
Ruh	*Ustilago hordei* (covered smut)	4
Rsp	*Ustilago hordei* (covered smut)	3
Rti	*Typhula incarnata* (gray snow mold)	1
Fb	*Fusarium* spp. (scab)	1
Ryd	BYDV (barley yellow dwarf virus)	2
Rym	BaYMV (barley yellow mosaic virus) BaMMV (barley mild mosaic virus)	13
Rsm	BSMV (barley stripe mosaic virus)	5
Rsg	*Schizaphis graminum* (green bug-aphid)	3
Rha	*Heterodera avenae* (cereal cyst nematode)	3

As a summary of reported QTL in barley (see Table 3.5), 757 QTL cover the whole barley genome for abiotic stress resistance, agronomic traits, biotic stress resistance, quality traits and others (Hayes *et al.* 2003).

Table 3.5 Summary of barley QTL report (stated by (Hayes *et al.* 2003))

Trait	Abiotic stress resistance	Agronomic traits	Biotic stress resistance	Quality traits	other
No. of phenotypes measured	26	58	15	27	5
No. of populations	7	16	10	8	3
No. of QTL	67	389	103	180	18

3.4 Contribution of wild barley to crop improvement

The genus *Hordeum* belongs to the tribe Triticeae, in the grass family Poaceae, and comprises two subspecies: *spontaneum* and *agriocrithon*. *H. spontaneum* is an annual plant with a short life cycle, diploid with only seven pairs of chromosomes, and self-pollinating. The genetic diversity of *H. spontaneum* has been identified by many markers, including isozyme polymorphisms (Nevo *et al.* 1979; Liu *et al.* 2002), RFLP-markers (Saghai-Maroof *et al.* 1984; Zhang *et al.* 1993), RAPD-markers (Dawson *et al.* 1993), SSR-markers (Saghai Maroof *et al.* 1994; Matus and Hayes 2002), AFLP-markers (Pakniyat *et al.* 1997; Turpeinen *et al.* 2003), and SNP-markers (Kanazin *et al.* 2002), respectively. *H. spontaneum* possesses more variation than cultivated barley, and many alleles are associated with specific environments (Forster *et al.* 2000; van-Rijn 2001). Distinct geographic patterns of genetic diversity are maintained in wild barley (*H. spontaneum*) despite migration (Morrell *et al.* 2003).

In ordinary plant breeding programs, new varieties are generated from a primary adapted pool of elite germplasm. In the past decades, intensive breeding of crop varieties by modern cultivation has further narrowed the gene pool, especially acute in self-pollinated crops (Tanksley *et al.* 1996a). Due to limited genetic variation among modern crops, efficient use of the genetic variation available in unadapted or wild relatives of modern cultivars is therefore essential to the continued improvement of cereal varieties (Tanksley and McCounch, 1997). In Europe the barley disease powdery mildew (*Erysiphe graminis*) that causes yield losses as high as 50 percent (Ellis 2002a) forces breeders to put their eyes on exploration of wild species.

The wild progenitor of cultivated barley, *H. spontaneum* has contributed many useful genes for several characters, especially diseases resistance to powdery mildew (Gustafsson and Claesson 1988) and leaf rust (Moseman *et al.* 1990). Many agronomic traits were investigated in this species, such as yield and its components (Nevo 1992; Ivandic *et al.* 2000; Ivandic *et al.* 2002) , floral structure (Giles and Bengtsson 1988), protein content (Jaradat 1991), spikelet weight (Volis *et al.* 2000), stem and spike length (Volis *et al.* 2002). Variation in physiological traits associated with salt tolerance (Forster *et al.* 1997), cold tolerance (Baum *et al.* 2003), drought tolerance and N-starvation (Robinson *et al.* 2000) has also been studied in *H. spontaneum*. Therefore, *H. spontaneum* is not only a rich source of new disease

resistance, but also an important species to offer genetic variability for economically important traits.

The generation of modern elite cultivars is a process based on decades of selections by breeders. The productivity, uniformity and quality of these cultivars are obvious differences from those of wild or unadapted germplasm. Therefore, once wild species carrying undesirable genes were applied into breeding plan, the negative effects followed with these genes - linkage drag - will be a considerable problem. In the past, breeding to introduce polygenic characters into a balanced population from wild species has been generally avoided. In order to make improvements of crops with unlimited resources of wild species and unadapted germplasm, it is necessary to find out an approach to reduce or break the linkage drags.

3.5 Advanced backcross-QTL analysis

Eshed and Zamir (1995) proposed to use variations of the backcross method (Wehrahn and Allard 1965) combined with genetic map information to map QTLs and select families with desired chromosomal regions.

With the development of the molecular markers and linkage map, Advanced backcross (AB) QTL mapping strategy (Tanksley and Nelson 1996) can be utilized to evaluate donor introgressions in the genetic background of an elite recurrent parent. Using this approach, favorable alleles and potentially valuable QTLs derived from either wild or adapted sources of germplasm and tagged with molecular markers can be associated with the performance of segregating offspring. In parallel, these QTL alleles will be transferred into near-isogenic lines (NILs) by means of marker associated selection breeding. Therefore, unlike the conventional QTL mapping methods, AB-QTL analysis can accelerate the process of marker-based breeding because the end products of analysis are close to NILs carrying favourable alleles.

Since the 1980s, Tanksley *et al.* (1996) has conducted genetic studies for fruit-size/shape and mapped 28 QTL of interesting traits using seven wild species of tomato and seven different crossing designs (Grandillo *et al.* 1999a). In Tanksley's lab, Alpert *et al.* (1995) reported a major QTL, *fw2.2*, controlling fruit weight that was found in a wild tomato species with a BC_1 population of 257 plants. In the next year, a favourable QTL allele (*fw9.1*) from the wild species was identified on chromosome 9, which increased fruit size by nearly 14% (Tanksley *et al.* 1996). This BC_1 population also was used to construct a genetic linkage map suitable for quantitative trait locus (QTL) analysis to be conducted in different backcross generations (Grandillo and Tanksley 1996). High resolution mapping and isolation of a YAC containing the site of *fw2.2* were accomplished (Alpert and Tanksley 1996). Besides controling the size of the developing tomato fruit, *fw2.2* also had secondary effects on fruit number and photosynthate distribution as a negative fruit-growth regulator (Nesbitt and Tanksley 2001; Liu *et al.* 2003). This was one of the first molecular characterizations of a locus that was originally identified entirely by QTL mapping, a landmark in QTL analysis. Moreover, *fs8.1*, a major QTL from wild species influencing fruit shape was characterized with the same population (Grandillo *et al.* 1996b). Its effect that could be traced with advanced backcross population (BC_4F_3) was identified to affect the fruit shape early in carpel development at least

6 days before anthesis with a set of NILs (Ku *et al.* 2000). At the same time, some NILs segregating for this region of interest were obtained. In other crossings of wild tomato species to cultivated tomato, hundreds of QTL were detected over different locations for 19 agronomic traits, including for tomato flavor (Fulton *et al.* 1997a; Bernacchi *et al.* 1998b; Grandillo *et al.* 1999b; Fulton *et al.* 2002). A larger number of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles were developed (Fulton *et al.* 1997b; Bernacchi *et al.* 1998a; Tanksley *et al.* 1998) and analyzed (Fulton *et al.* 2000; Hanson *et al.* 2000; Monforte and Tanksley 2000).

Since the first report in tomato (Tanksley *et al.* 1996), AB-QTL analysis has been successfully applied in many crops to detect and transfer valuable QTLs from unadapted germplasm into elite breeding lines, such as in rice (Xiao *et al.* 1998; Yamamoto *et al.* 1998; Yamamoto *et al.* 2000; Moncada *et al.* 2001; Li *et al.* 2002; Lin *et al.* 2003; Takeuchi *et al.* 2003; Thomson *et al.* 2003), and in maize (Ho *et al.* 2002). Recently, the first two AB-QTL studies in wheat and barley were published by (Huang *et al.* 2003) and (Pillen *et al.* 2003), respectively.

4 Mapping of new microsatellite markers

4.1 Introduction

Despite extensive mapping efforts of the barley genome described in chapter 3.2.2.5, large gaps are still present in the PCR-based barley microsatellite map and make the coverage quite uneven because of clustering around some centromeres. To enhance the marker density of existing genetic maps of barley (*Hordeum vulgare* L.), a new set of microsatellite markers containing dinucleotide motifs was developed from genomic clones by Dr. Tatjana Sjakste. Here, I present their integration into previously published maps. In total, 127 functional microsatellite markers were mapped onto the seven barley chromosomes using two mapping populations and/or wheat-barley chromosome addition-lines. The polymorphism information (PIC) for these markers ranged from 0.05 to 0.94 with an average of 0.60. The number of alleles per locus varied from 1 to 9. On average, 3.9 alleles per primer pair were screened.

4.2 Materials and methods

4.2.1 Molecular markers

The barley variety Franka was used as the genomic DNA source for the identification and isolation of microsatellites (Li *et al.* 2003). A total of 254 primer pairs flanking the microsatellite sites were designed using the computer program PRIMER v.0.5. Mapped barley microsatellites isolated from the genomic library were designed as GBMS for 'Gatersleben Barley MicroSatellite' and numbered consecutively. A small letter was added after the number if a microsatellite identified more than one locus. The GBMS primers were developed by Dr. Tatjana Sjakste and provided by Dr. Martin Ganal.

Besides the newly developed GBMS microsatellites, 36 microsatellites described in Ramsay *et al.* (2000) were used in the S/M or I/F maps as reference markers.

4.2.2 Plant materials

The first population was the principal mapping population of the North American Barley Genome Mapping Project, constructed from the cross of 'Steptoe' × 'Morex' (S/M), and consisted of 80 doubled haploids (Kleinhofs *et al.* 1993).

The second population contained 65 DH plants derived from the cross of the winter barley cultivars 'Igri' × 'Franka' (I/F) by Foroughi-Wehr and Friedt (1984).

The microsatellite markers were tested for functionality and polymorphism against a panel of nine barley lines: the *H. spontaneum* lines, HS213, HS277, and HS584, and the varieties 'Brenda', 'Trasco', 'Steptoe', 'Morex', 'Igri', and 'Franka'.

A set of wheat (cv. 'Chinese Spring') / barley (cv. 'Betzes') chromosome addition lines was used to confirm the assignment of new microsatellites to the seven chromosomes in barley (Islam *et al.* 1981). The addition lines for chromosomes 3H, 4H, 5H, 6H and 7H were disomic, the addition lines for 2HS and 2HL ditelosomic, respectively. Microsatellites which were not polymorphic in the two mapping populations were assigned to specific chromosomes and to specific arms of chromosomes using these addition lines. Aliquots of genomic DNA of both mapping populations and the addition lines were kindly provided by Prof. Dr. A. Graner (IPK Gatersleben).

4.2.3 Plant DNA extraction

DNA extraction buffer		TE buffer	
Tris-HCl pH 8.0	100 mM	Tris-HCl pH 8.0	10 mM
NaCl	500 mM	EDTA pH 8.0	1 mM
EDTA pH 8.0	50 mM		
SDS	1.25 %		
Nabisulfite	3.8 g/l		

Total genomic DNA was isolated from leaf tissue according to Anderson *et al.* (1993). Approximately 20-30 g homogenized barley leaf tissue was grinded in a well chilled mortar with pestle in liquid nitrogen. With a chilled paintbrush, the fine powder was transferred into a chilled 50 ml propylene tube. Twenty-five ml extraction buffer at 65 °C was added to approximately 15 ml frozen fine powder in the tube and incubated at 65 °C for 40-60 min and shaken each 10 min. Subsequently, 15 ml of chloroform : isoamylalcohol (24 : 1) were added to the tube and mixed by shaking until a good emulsion was formed. After centrifugation for 15 min. at 3,000 rpm, the aqueous phase was transferred into a new tube. Usually, this step of the chloroform extraction was repeated. After mixing with 2 volumes of ice-cold 95% ethanol, the DNA was precipitated and hooked out with a glass hook into a 15 ml propylene tube containing cold 70% Ethanol. After purification with Ethanol, the DNA was airdried and dissolved at 50-60 °C in 1-3 ml of TE buffer for 40 min., and was stored at 4 °C. The DNA concentration is approximately 300-500 µg/ml.

4.2.4 PCR reaction

PCR reaction mixture		PCR reaction conditions		
Tris-HCl	10 mM	Pre-denaturation	94°C	2 min.
KCl	50 mM	45 cycles of		
$MgCl_2$	1.5 mM	Denaturation	94°C	1 min.
dNTP	0.2 mM	Annealing	55°C, 58°C, or 60°C	1 min.
Taq DNA pulymerase	1 u	Extension	72°C	2 min.
Primer pairs	250 nM	Extension	72°C	7 min.
DNA template	50-150 ng	Incubation	12°C	

PCR reaction was carried out by a GeneAmp® PCR system 9700 (Perkin-Elmer, Norwalk, CT) with a 25 µl reaction volume for each sample.

4.2.5 Analysis of PCR products

Since one primer of each pair was labeled with fluorescent Cy5, all analysis of PCR products was performed on the electrophoresis and detection system ALF*express*®II DNA Analyser (Amersham Biosciences) with denaturing 6% polyacrylamide gels (ReproGel™) in short gel cassettes. The fragment size calculation was performed by using internal size standards and the program Fragment Analyser 1.2 (Amersham Biosciences).

4.2.6 Analysis and mapping of microsatellites

The segregation data for the polymorphic microsatellites in S/M and I/F populations were tested for segregation distortion against the expected 1:1 ratio by calculating chi-square values using Joinmap (version 3.0, Stan P. and van Ooijen J.W., CPRO-DLO, Wageningen, The Netherlands).

The software package MAPMAKER/Exp version 3.0b (Lander *et al.* 1987) was then used to construct genetic maps in the two mapping populations. Markers mapping to the same linkage group were considered as linked if they showed a LOD score of at least 3.0. The final order of markers in each chromosome was confirmed with the 'Ripple' command. Recombination fractions were converted to centiMorgans with the Kosambi mapping function (Kosambi 1944). The data for the framework of RFLP makers were kindly provided by Prof. Dr. A. Graner (IPK Gatersleben).

In order to measure the informativeness of a DNA marker in the analyzed material, the polymorphic information content (PIC) for each loci was calculated according to Weber (1990) and Anderson *et al.* (1993).

4.3 Results

4.3.1 Screening for polymorphism and diversity analysis

A total of 254 newly developed primers paris flanking dinucleotide microsatellites (obtained from Dr. M. Ganal) were used for quality checking and analysis of diversity in barley. The amplification quality of these newly developed primers was evaluated on a panel of six barley varieties and three *H. spontaneum* lines according to the classification by Pepin *et al.* (1995) and Smulders *et al.* (1997). Of the 254 microsatellites tested, 95 amplified well scorable fragments consisting of one expected strong fragment while 72 amplified a weaker fragment of the expected size or relatively strong stutter bands. Denaturing-PAGE electrophoresis in combination with a laser fluorescence sequencer yielded a high resolution of the products differing in steps of two base pairs. In order to determine the informativeness of the microsatellites, the PIC values were calculated. They ranged from 0.05 to 0.94 with on average 0.60 (Table 4.1). The number of alleles ranged from one to nine with an average of 3.9 alleles per locus. Only three microsatellites were monomorphic on the test panel. No significant correlation between the PIC value and number of repeats was detected. Out of the 254 primer pairs, 86 (34%) and 76 (30%) detected polymorphism between two parental lines of I/F and S/M, respectively. Forty-seven (19%) primer pairs were polymorphic in both mapping populations. Few primer pairs amplified additional monomorphic or polymorphic fragments in addition to the expected microsatellite fragment. The microsatellites GBMS128, GBMS203, GBMS219 and GBMS223 produced two or more polymorphic loci, which could be mapped.

Table 4.1 List of barley microsatellites including repeat motif, PCR conditions, PIC, allele numbers and chromosomal location

Marker	Motif types	Ann. Tem.	PIC	Alleles	Expected sizes	S/M[1]	I/F[2]	W/B[3]
GBMS2	$(GA)_{14}$	60	0.79	6	110	2H		
GBMS3	$(AG)_{14}$	60	0.67	4	150	7H		7H
GBMS9	$(GA)_{23}$	60		1	190			3H
GBMS11	$(AG)_{14}$	60	0.75	2	208		2H	
GBMS12	$(CT)_{11}(CA)_{20}$	60	0.85	4	248	1H		
GBMS13	$(CT)_{10}$	60	0.59	3	131		5H	
GBMS14	$(CT)_{15}$	60	0.83	5	142		1H	
GBMS15	$(GT)_{19}$	60	0.49	3	209	4H	4H	
GBMS17	$(TG)_{11}$	60	0.32	4	133		1H	
GBMS20	$(TC)_{6}$	60	0.54	2	163			3H
GBMS22	$(GT)_{20}$	60	0.72	5	116	3H		
GBMS28	$(GT)_{11}$	60	0.26	5	142		4H	4H
GBMS29	$(AC)_{12}(AT)_{14}$	60	0.84	7	147		4H	
GBMS31	$(AG)_{14}$	60	0.44	3	113	2H	2H	
GBMS32	$(GT)_5CT(GT)_{11}$	60	0.64	5	113	5H		5H
GBMS33	$(AT)_5AG(AC)_{19}(AT)_7$	60	0.28	5	112	6H	6H	

Marker	Motif types	Ann. Tem.	PIC	Alleles	Expected sizes	S/M[1]	I/F[2]	W/B[3]
GBMS35	$(GA)_{12}$	60	0.75	5	145	7H		
GBMS37	$(TG)_{27}$	60	0.41	7	144	1H	1H	
GBMS38	$(TC)_{22}$	60	0.83	5	147		3H	
GBMS40	$(AG)_{18}$	55	0.23	3	193	2H		
GBMS41	$(GT)_{25}$	60	0.93	3	193	7H		
GBMS45	$(AC)_{11}$	60	0.72	5	150		3H	3H
GBMS46	$(AG)_{32}$	60	0.60	5	205	3H		3H
GBMS48	$(AG)_8CA(AG)_{10}$	60	0.72	4	182	3H		3H
GBMS49	$(AG)_{14}$	60	0.72	5	138		4H	
GBMS50	$(AC)_{13}(AG)_{14}$	60	0.72	5	133	3H	3H	3H
GBMS53	$(GT)_{11}$	60	0.36	5	111	1H	1H	
GBMS54	$(CA)_9$	60	0.69	4	100	1H		
GBMS56	$(AC)_{10}$	60	0.20	2	181			6H
GBMS57	$(CT)_9$	60	0.49	2	149		3H	3H
GBMS58	$(CA)_7$	60	0.44	2	106			6H
GBMS60	$(GT)_{11}$	55	0.64	3	166		5H	5H
GBMS61	$(TG)_{10}$	60	0.68	3	179			7H
GBMS62	$(AG)_{10}$	60	0.68	5	126		1H	
GBMS63	$(AC)_{11}$	60	0.60	6	185	7H	7H	7H
GBMS65	$(AG)_{10}$	60	0.35	2	105	1H		
GBMS66	$(CA)_{10}$	60	0.65	5	113		2H	
GBMS67	$(AT)_8(GA)_{12}$	60	0.11	3	142			5H
GBMS68	$(CT)_{11}$	60	0.53	3	154		5H	
GBMS69	$(TC)_{12}$	60	0.38	2	118			2HS
GBMS70	$(AT)_8(GA)_{12}$	60	0.51	2	150	5H		
GBMS72	$(TG)_{11}$	60	0.73	5	140		6H	6H
GBMS74	$(CA)_{22}$	60	0.77	7	149	3H	3H	3H
GBMS75	$(AT)_8(GT)_{16}$	55	0.94	5	193	5H	5H	
GBMS77	$(GT)_{18}$	60	0.84	7	182		5H	5H
GBMS79	$(GT)_{11}$	60	0.52	4	160			4H
GBMS81	$(AG)_{12}$	60	0.65	4	174		4H	4H
GBMS83	$(AC)_{20}(AT)_5$	60	0.59	3	144	6H	6H	
GBMS87	$(CT)_{15}$	60	0.74	6	167	4H		4H
GBMS88	$(GT)_8(GC)_6G_4(GT)_6$	60	0.44	2	221	7H	7H	7H
GBMS89	$(GA)_9$	60	0.69	4	125	3H		3H
GBMS90	$(GA)_{17}$ GTGT$(G)_{11}$	60	0.95	4	111		2H	
GBMS93	$(CT)_{12}$	60	0.05	3	177		1H	
GBMS94	$(GT)_{11}$	60	0.79	2	125	7H		
GBMS95	$(CT)_4CCA(CT)_{10}$	55	0.64	3	104	2H		
GBMS96	$(GA)_{11}$	60	0.35	2	136			4H
GBMS102	$(CA)_{10}$	60	0.52	4	143	3H		
GBMS103	$(GT)_{19}$	60	0.52	4	188	2H		
GBMS105	$(CT)_{17}(AT)_{29}$	60	0.84	5	141			3H
GBMS106	$(GA)_{38}$	60	0.84	7	221		5H	
GBMS107	$(GT)_8T(GT)_4$	60	0.62	4	189		6H	
GBMS110	$(GA)_{21}$	60	0.67	4	141	3H		3H
GBMS111	$(CA)_{15}$	60	0.74	5	150	7H		
GBMS112	$(CA)_{20}(AT)_{23}$	60	0.07	4	239		7H	
GBMS114	$(CT)_9$	60	0.62	5	117	4H	4H	4H
GBMS115	$(AT)_{38}$	55	0.84	2	183	5H		
GBMS117	$(CT)_9$	60	0.58	2	149		3H	3H
GBMS119	$(CA)_{22}$	60	0.68	4	114	5H		5H
GBMS120	$(AT)_{35}$	60	0.83	5	230		7H	
GBMS121	$(GA)_{13}$	60	0.47	5	185	6H		
GBMS125	$(GA)_6$	60	0.49	2	128	6H		
GBMS128a	$(GT)_{12}$	55	0.79	3	140		4H	
GBMS128b		55	0.74	4			2H	
GBMS129	$(CA)_{11}$	55	0.69	4	153	7H		7H
GBMS133	$(CA)_{17}(AT)_{12}$	55	0.74	6	184	4H		4H
GBMS135	$(GT)_{23}$	55	0.75	6	139	6H		

Marker	Motif types	Ann. Tem.	PIC	Alleles	Expected sizes	S/M[1]	I/F[2]	W/B[3]
GBMS137	$(GA)_{40}$	55	0.86	8	207	2H		2H
GBMS138	$(GT)_{11}$	55	0.52	4	156		3H	3H
GBMS139	$(CA)_{10}$	58	0.85	6	145	7H		7H
GBMS140	$(CA)_{19}(AT)_{15}$	58	0.89	6	210	3H	3H	3H
GBMS141	$(CA)_{16}$	60	0.79	4	147	7H	7H	
GBMS143	$(CA)_{8}$	60	0.79	4	196	1H		
GBMS147	$(GT)_{18}$	60	0.20	2	130	3H		3H
GBMS149	$(CAT)_{24}$	60	0.64	5	110	3H	3H	3H
GBMS150	$(CT)_{12}$	60	0.35	3	118	4H	4H	
GBMS154	$(CAT)_{7}$	60	0.74	5	116			5H
GBMS156	$(GT)_{11}$	60	0.69	4	182	5H		
GBMS157	$(GT)_{10}$	60	0.54	2	141	3H		3H
GBMS160	$(GA)_{14}$	60	0.72	5	196	2H		
GBMS163	$(GT)_{15}$	60	0.37	3	165			3H
GBMS164	$(GT)_{16}$	60	0.48	4	109	7H		
GBMS166	$(CA)_{6}TCGCT(CA)_{9}$	60	0.74	5	156		3H	3H
GBMS174	$(GT)_{19}$	60	0.38	2	131		5H	5H
GBMS178	$(GT)_{11}$	60	0.05	5	136	6H		
GBMS180	$(GA)_{30}$	60	0.85	9	240	6H		6H
GBMS183	$(GT)_{5}(CT)_{3}(GT)_{8}$	55	0.57	5	185	7H		7H
GBMS184	$(GT)_{5}(GA)_{14}$	60	0.62	4	148	1H		
GBMS185	$(GA)_{14}$	55	0.51	4	130	3H		
GBMS187	$(GT)_{20}$	60	0.94	2	143		1H	
GBMS188	$(GA)_{19}$	55	0.68	5	147	2H		
GBMS189	$(GA)_{32}$	60	0.64	5	145	3H		3H
GBMS190	$(GA)_{10}$	60	0.79	2	130		4H	4H
GBMS192	$(GT)_{14}$	60	0.47	5	199	7H		7H
GBMS196	$(GT)_{10}$	60	0.49	3	183	5H		5H
GBMS198	$(CA)_{4}GA(CA)_{3}(GA)_{6}$	60	0.57	3	146	3H		3H
GBMS201	$(GT)_{15}$	60	0.35	2	106	6H		
GBMS202	$(GT)_{12}$	60	0.89	3	140	2H		
GBMS203a	$(GT)_{14}$	60	0.06	5	168	3H		3H
GBMS203b		60	0.07	2		4H		4H
GBMS204	$(GT)_{10}$	60	0.64	3	150		3H	3H
GBMS206	$(GA)_{14}$	60		1	184			3H
GBMS211	$(GT)_{10}$	60	0.35	2	126			4H
GBMS212	$(GA)_{14}$	60	0.49	3	152	3H		3H
GBMS214	$(GT)_{10}$	60	0.37	3	119			4H
GBMS216	$(GA)_{33}$	60	0.77	5	229		2H	
GBMS219a	$(GT)_{14}$	60	0.84	2	128	1H		
GBMS219b		60	0.28	5	108	5H		5H
GBMS219c		60	0.51	2	112	7H		7H
GBMS219d		60	0.23	2		5H		
GBMS222	$(AC)_{3}TC(AC)_{8}$	60	0.51	2	171	6H		6H
GBMS223a	$(CA)_{10}$	60	0.30	3	144			7H
GBMS223b		60	0.91	4	166	3H		3H
GBMS226	$(GT)_{9}$	60	0.63	3	188		7H	7H
GBMS229	$(GT)_{10}$	60	0.67	4	156	2H		
GBMS230	$(GT)_{9}$	60	0.72	8	150	2H		
GBMS233	$(TG)_{8}T_{3}(GT)_{3}$	60	0.79	3	129		2H	
GBMS235	$(TC)_{12}T(TC)_{23}$	60	0.80	5	142		2H	
GBMS238	$(CA)_{12}$	60		1	184			6H
GBMS240	$(GA)_{10}$	60	0.73	4	147	7H		
GBMS244	$(GT)_{10}$	60	0.64	3	243		2H	
GBMS245	$(CA)_{11}\ldots(CA)_{14}$	60	0.74	3	231			2H
GBMS247	$(GT)_{9}$	60	0.72	5	228	2H		
GBMS254	$(GT)_{12}$	60	0.94	2	161	7H		

[1] Chromosomal location by linkage mapping on the population Steptoe/Morex
[2] Chromosomal location by linkage mapping on the population Igri/Franka
[3] Chromosomal location on wheat-barley chromosome addition lines

4.3.2 Mapping of microsatellite loci

Of the 127 loci mapped in the progeny of S/M, 6 (5%) and 15 (12%) deviated significantly at $P < 0.1$ and $P < 0.05$ from the 1:1 allele frequency, respectively. Eighteen loci were skewed toward 'Morex' whereas three were skewed toward 'Steptoe' (see Table 4.2). In contrast to the low level of segregation distortion in the S/M population, 32 (43%) loci significantly deviated ($P < 0.1$) from the expected ratio 1:1 in the I/F DH population, 12 (16%) were at $P < 0.0001$ significance level (see Table 4.3). Out of the 32 SSR loci, 25 were skewed toward 'Igri' while seven were toward 'Franka'. Overall, 127 primer pairs resulting in 133 microsatellite loci were identified their linkage groups in barley. In total, 115 loci included 78 integrated in the S/M map and 53 loci in the I/F map, while 16 loci were integrated in both maps. In all cases, the mapping positions corresponded in the two maps. The assignment of 48 mapped loci was reconfirmed by a set of wheat-barley chromosome addition lines. In addition, 18 microsatellites/loci that were monomorphic in the two mapping populations were assigned to chromosomes by this method (See Table 4.1). In order to facilitate the comparison with the microsatellite map produced by (Ramsay *et al.* 2000) 29 previously mapped microsatellites were integrated into the S/M map and 9 microsatellites into the I/F map. The total map deduced contained 204 loci for S/M and 105 loci for I/F including the framework markers. (Fig. 4.1)

Table 4.2 Frequency distributions per locus in S/M mapping population

	Locus	Plants	Morex	Steptoe	χ^2 (1:1)	*P*-value	Significance
1	Bmac18	80	38	42	0.20	> 0.100	-
2	Bmac31	78	41	37	0.21	> 0.100	-
3	Bmac32	78	37	41	0.21	> 0.100	-
4	Bmac40	76	42	34	0.84	> 0.100	-
5	Bmac90	78	33	45	1.85	> 0.100	-
6	Bmac163	79	51	28	6.70	0.005-0.010	***
7	Bmac213	80	29	51	6.05	0.010-0.025	**
8	Bmac303	80	52	28	7.20	0.005-0.010	***
9	Bmac316	77	31	46	2.92	0.050-0.100	*
10	Bmac602	79	43	36	0.62	> 0.100	-
11	Bmag120	80	37	43	0.45	> 0.100	-
12	Bmag125	79	45	34	1.53	> 0.100	-
13	Bmag135	80	38	42	0.20	> 0.100	-
14	Bmag138	80	35	45	1.25	> 0.100	-
15	Bmag140	80	43	37	0.45	> 0.100	-
16	Bmag187	77	37	40	0.12	> 0.100	-
17	Bmag211	80	35	45	1.25	> 0.100	-
18	Bmag223	79	49	30	4.57	0.025-0.050	**
19	Bmag225	80	39	41	0.05	> 0.100	-
20	Bmag341	80	42	38	0.20	> 0.100	-
21	Bmag387	79	52	27	7.91	< 0.005	****
22	Bmag490	78	39	39	0.00	> 0.100	-
23	Bmag507	80	42	38	0.20	> 0.100	-
24	Bmag518	80	46	34	1.80	> 0.100	-
25	Bmag579	80	40	40	0.00	> 0.100	-
26	Bmag603	79	35	44	1.03	> 0.100	-

	Locus	Plants	Morex	Steptoe	χ^2 (1:1)	*P*-value	Significance
27	Bmag606	78	43	35	0.82	> 0.100	-
28	Bmag613	80	38	42	0.20	> 0.100	-
29	EBmac541	79	42	37	0.32	> 0.100	-
30	EBmac603	80	39	41	0.05	> 0.100	-
31	EBmac635	79	43	36	0.62	> 0.100	-
32	EBmac679	80	43	37	0.45	> 0.100	-
33	EBmac701	79	42	37	0.32	> 0.100	-
34	EBmac713	79	37	42	0.32	> 0.100	-
35	EBmac715	79	47	32	2.85	0.050-0.100	*
36	EBmac788	79	42	37	0.32	> 0.100	-
37	EBmac806	80	42	38	0.20	> 0.100	-
38	EBmac824	79	36	43	0.62	> 0.100	-
39	EBmac906	77	41	36	0.32	> 0.100	-
40	EBmag705	79	38	41	0.11	> 0.100	-
41	GBMS2	79	39	40	0.01	> 0.100	-
42	GBMS3	79	41	38	0.11	> 0.100	-
43	GBMS12	77	40	37	0.12	> 0.100	-
44	GBMS15	72	41	31	1.39	> 0.100	-
45	GBMS22	77	36	41	0.32	> 0.100	-
46	GBMS31	74	38	36	0.05	> 0.100	-
47	GBMS32	76	47	29	4.26	0.025-0.050	**
48	GBMS33	80	36	44	0.80	> 0.100	-
49	GBMS35	78	38	40	0.05	> 0.100	-
50	GBMS37	74	33	41	0.86	> 0.100	-
51	GBMS38	80	48	32	3.20	0.050-0.100	*
52	GBMS40	54	19	35	4.74	0.025-0.050	**
53	GBMS41	78	37	41	0.21	> 0.100	-
54	GBMS46	75	32	43	1.61	> 0.100	-
55	GBMS48	79	35	44	1.03	> 0.100	-
56	GBMS50	76	33	43	1.32	> 0.100	-
57	GBMS53	78	41	37	0.21	> 0.100	-
58	GBMS54	77	37	40	0.12	> 0.100	-
59	GBMS63a	80	60	20	20.00	< 0.0001	*******
60	GBMS63b	80	42	38	0.20	> 0.100	-
61	GBMS65	78	34	44	1.28	> 0.100	-
62	GBMS70	77	48	29	4.69	0.025-0.050	**
63	GBMS74	77	38	39	0.01	> 0.100	-
64	GBMS75a	80	52	28	7.20	0.005-0.010	***
65	GBMS75b	78	44	34	1.28	> 0.100	-
66	GBMS83	73	33	40	0.67	> 0.100	-
67	GBMS85	79	39	40	0.01	> 0.100	-
68	GBMS87	77	37	40	0.12	> 0.100	-
69	GBMS88	78	36	42	0.46	> 0.100	-
70	GBMS89	78	35	43	0.82	> 0.100	-
71	GBMS94	80	38	42	0.20	> 0.100	-
72	GBMS95	79	47	32	2.85	0.050-0.100	*
73	GBMS102	79	35	44	1.03	> 0.100	-
74	GBMS103	79	48	31	3.66	0.100-0.050	*
75	GBMS105	79	66	13	35.56	< 0.0001	*******
76	GBMS107	79	37	42	0.32	> 0.100	-
77	GBMS110	79	33	46	2.14	> 0.100	-
78	GBMS111	79	42	37	0.32	> 0.100	-
79	GBMS114	79	40	39	0.01	> 0.100	-
80	GBMS115	80	51	29	6.05	0.010-0.025	**
81	GBMS119	77	38	39	0.01	> 0.100	-
82	GBMS121	79	37	42	0.32	> 0.100	-
83	GBMS125	79	35	44	1.03	> 0.100	-
84	GBMS129	79	41	38	0.11	> 0.100	-
85	GBMS133	75	44	31	2.25	> 0.100	-
86	GBMS135	78	37	41	0.21	> 0.100	-

	Locus	Plants	Morex	Steptoe	χ^2 (1:1)	*P*-value	Significance
87	GBMS137	77	42	35	0.64	> 0.100	-
88	GBMS139	78	43	35	0.82	> 0.100	-
89	GBMS140	77	34	43	1.05	> 0.100	-
90	GBMS141	78	38	40	0.05	> 0.100	-
91	GBMS143	78	38	40	0.05	> 0.100	-
92	GBMS147	80	37	43	0.45	> 0.100	-
93	GBMS149	76	36	40	0.21	> 0.100	-
94	GBMS150	76	40	36	0.21	> 0.100	-
95	GBMS156	80	50	30	5.00	0.010-0.025	**
96	GBMS157	80	34	46	1.80	> 0.100	-
97	GBMS160	80	44	36	0.80	> 0.100	-
98	GBMS164	80	42	38	0.20	> 0.100	-
99	GBMS178	79	38	41	0.11	> 0.100	-
100	GBMS180	79	38	41	0.11	> 0.100	-
101	GBMS183	79	31	48	3.66	0.050-0.100	*
102	GBMS184	79	37	42	0.32	> 0.100	-
103	GBMS185	79	34	45	1.53	> 0.100	-
104	GBMS188	79	44	35	1.03	> 0.100	-
105	GBMS189	77	34	43	1.05	> 0.100	-
106	GBMS192	79	34	45	1.53	> 0.100	-
107	GBMS194	75	43	32	1.61	> 0.100	-
108	GBMS196	75	48	27	5.88	0.010-0.025	**
109	GBMS198	79	35	44	1.03	> 0.100	-
110	GBMS201	80	38	42	0.20	> 0.100	-
111	GBMS202	79	45	34	1.53	> 0.100	-
112	GBMS203a	78	35	43	0.82	> 0.100	-
113	GBMS203b	80	43	37	0.45	> 0.100	-
114	GBMS212	79	39	40	0.01	> 0.100	-
115	GBMS219a	80	34	46	1.80	> 0.100	-
116	GBMS219b	79	38	41	0.11	> 0.100	-
117	GBMS219c	79	42	37	0.32	> 0.100	-
118	GBMS219d	80	38	42	0.20	> 0.100	-
119	GBMS222	76	37	39	0.05	> 0.100	-
120	GBMS223a	79	64	15	30.39	< 0.0001	*******
121	GBMS223b	79	35	44	1.03	> 0.100	-
122	GBMS229	78	39	39	0.00	> 0.100	-
123	GBMS230	78	42	36	0.46	> 0.100	-
124	GBMS240	79	41	38	0.11	> 0.100	-
125	GBMS247	77	35	42	0.64	> 0.100	-
126	GBMS254	80	43	37	0.45	> 0.100	-
127	WMC1E8	80	40	40	0.00	> 0.100	-

Significance levels:
*:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001 ******:0.0005 *******:0.0001

Table 4.3 Frequency distributions per locus in I/F mapping population

	Locus	Plants	Franka	Igri	χ^2 (1:1)	P-value	Significance
1	Bmac31	63	23	40	4.59	0.025-0.050	**
2	Bmac134	64	36	28	1.00	> 0.100	-
3	Bmac213	64	24	40	4.00	0.025-0.050	**
4	Bmac382	65	33	32	0.02	> 0.100	-
5	Bmag7	63	31	32	0.02	> 0.100	-
6	Bmag125	65	41	24	4.45	0.025-0.050	**
7	Bmag138	65	36	29	0.75	> 0.100	-
8	Bmag206	65	32	33	0.02	> 0.100	-
9	Bmag223	63	20	43	8.40	0.001-0.005	****
10	Bmag500	57	21	36	3.95	0.025-0.050	**
11	EBmac906	65	30	35	0.38	> 0.100	-
12	EBmac603	65	35	30	0.38	> 0.100	-
13	EBmac755	64	27	37	1.56	> 0.100	-
14	GBMS3	65	24	41	4.45	0.025-0.050	**

	Locus	Plants	Franka	Igri	χ^2 (1:1)	P-value	Significance
15	GBMS11	65	43	22	6.78	0.005-0.010	***
16	GBMS13	65	34	31	0.14	> 0.100	-
17	GBMS14	63	31	32	0.02	> 0.100	-
18	GBMS15	64	33	31	0.06	> 0.100	-
19	GBMS17	65	33	32	0.02	> 0.100	-
20	GBMS28	65	26	39	2.60	> 0.100	-
21	GBMS29	64	30	34	0.25	> 0.100	-
22	GBMS31	64	37	27	1.56	> 0.100	-
23	GBMS33	65	29	36	0.75	> 0.100	-
24	GBMS37	62	34	28	0.58	> 0.100	-
25	GBMS38	60	28	32	0.27	> 0.100	-
26	GBMS45	62	16	46	14.52	0.0001-0.0005	******
27	GBMS46	64	7	57	39.06	< 0.0001	*******
28	GBMS49	64	30	34	0.25	> 0.100	-
29	GBMS50	63	7	56	38.11	< 0.0001	*******
30	GBMS53	63	25	38	2.68	> 0.100	-
31	GBMS57	64	8	56	36.00	< 0.0001	*******
32	GBMS60	65	33	32	0.02	> 0.100	-
33	GBMS62	63	28	35	0.78	> 0.100	-
34	GBMS63	65	22	43	6.78	0.005-0.010	***
35	GBMS66	65	43	22	6.78	0.005-0.010	***
36	GBMS68	63	32	31	0.02	> 0.100	-
37	GBMS72	62	21	41	6.45	0.010-0.025	**
38	GBMS74	65	14	51	21.06	< 0.0001	*******
39	GBMS75	65	22	43	6.78	0.005-0.010	***
40	GBMS77	62	32	30	0.06	> 0.100	-
41	GBMS81	65	30	35	0.38	> 0.100	-
42	GBMS83	63	25	38	2.68	> 0.100	-
43	GBMS85	59	49	10	25.78	< 0.0001	*******
44	GBMS88	19	6	13	2.58	> 0.100	-
45	GBMS89	65	8	57	36.94	< 0.0001	*******
46	GBMS90	64	42	22	6.25	0.010-0.025	**
47	GBMS93	64	32	32	0.00	> 0.100	-
48	GBMS106	65	25	40	3.46	0.050-0.100	*
49	GBMS107	65	27	38	1.86	> 0.100	-
50	GBMS112	65	32	33	0.02	> 0.100	-
51	GBMS114	65	31	34	0.14	> 0.100	-
52	GBMS117	65	8	57	36.94	< 0.0001	*******
53	GBMS120	64	22	42	6.25	0.010-0.025	**
54	GBMS128	63	36	27	1.29	> 0.100	-
55	GBMS128b	65	29	36	0.75	> 0.100	-
56	GBMS135	62	24	38	3.16	0.050-0.100	*
57	GBMS137	53	27	26	0.02	> 0.100	-
58	GBMS138	65	30	35	0.38	> 0.100	-
59	GBMS139	62	6	56	40.32	< 0.0001	*******
60	GBMS140	62	8	54	34.13	< 0.0001	*******
61	GBMS141	54	13	41	14.52	0.0005-0.001	******
62	GBMS149	62	6	56	40.32	< 0.0001	*******
63	GBMS150	64	31	33	0.06	> 0.100	-
64	GBMS166	65	8	57	36.94	< 0.0001	*******
65	GBMS174	64	20	44	9.00	0.005-0.010	****
66	GBMS187	65	34	31	0.14	> 0.100	-
67	GBMS190	65	33	32	0.02	> 0.100	-
68	GBMS200	65	37	28	1.25	> 0.100	-
69	GBMS204	63	15	48	17.29	< 0.0001	*******
70	GBMS216	59	24	35	2.05	> 0.100	-
71	GBMS226	62	32	30	0.06	> 0.100	-
72	GBMS233	64	43	21	7.56	0.005-0.010	***
73	GBMS235	65	41	24	4.45	0.025-0.050	**
74	GBMS244	56	33	23	1.79	> 0.100	-

	Locus	Plants	Franka	Igri	χ^2 (1:1)	P-value	Significance
75	GMS21	62	25	37	2.32	> 0.100	-

Significance levels:
*:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001 ******:0.0005 *******:0.0001

4.4 Discussion

A total of 167 microsatellite markers (66%) were classified as functional and amplified fragments of the expected size based on sequence data. Nonfunctional primers amplified either a smear, monomorphic fragments of the wrong size, many fragments or nothing. A proportion of 66% functional primers is in agreement with results obtained for wheat using libraries enriched for single and low copy sequences by predigestion with a methylation sensitive restriction enzyme (Röder *et al.* 1998). For primer pairs derived of libraries enriched for microsatellites, a level of functionality of 83% was reported based on tests with agarose gels (Ramsay *et al.* 2000) which is not directly comparable to the analysis on DNA sequence analyzers. In Ramsay *et al*. (2000) 488 (83%) out of 585 tested primer pairs were classified as functional, while only 242 primer pairs (41%) were genetically mapped. From the primer pairs described in this research out of 254 tested primer pairs 167 (66%) were classified as functional, but 127 primer pairs (50%) resulting in 133 loci could be mapped or assigned to chromosomes.

According to literature data, the microsatellites developed from genomic DNA libraries generally have longer repeat sequences and were more polymorphic than those from ESTs. In rice, Cho *et al*. (2000) provided evidentces and demonstrated cDNA-derived microsatellites with lower variability values than those isolated from genomic DNA, including number of alleles, allele size range and expected genetic diversity. Pillen *et al*. (2000) reported an average PIC value of 0.38 for EST-derived barley microsatellites, a similar value of 0.45 was reported by Thiel *et al*. (2003). However, both PIC values of barley genomic SSRs gained in this study and construction of the first SSRs map by Ramsay *et al*. (2000) were 0.60 and 0.58, respectively.

A high level of segregation distortion was also observed with RFLPs in the I/F mapping population and has possibly been caused during the development of the doubled haploids (Graner *et al.* 1991). Deviations from the expected Mendelian segregation ratios were previously reported for mapping in many different plants based on both codominant and dominant marker types (Lyttle 1991; Xu *et al.* 1997; Temnykh *et al.* 2000; Chani *et al.* 2002; Knox and Ellis 2002). In a study of SSR mapping in maize (Sharopova *et al.* 2002), no evidence was found that the order of markers was influenced by distorted segregation. These findings were consistent with a previous report for *Arabidopsis* (Liu *et al.* 1996a).

Clustering of microsatellites was observed around the centromeres especially on chromosomes 2H, 3H, 6H and 7H. Strong centromeric clustering was also described in the microsatellite map of Ramsay *et al.* (2000) and is most likely the result of suppressed recombination around the centromeres as it has been described in the physical map of Künzel *et al.* (2000). No significant clusters of SSR markers were detected in centromeric or telomeric regions of the high-density genetic map of rice (Temnykh *et al.* 2000).

The improved coverage of the barley genetic map with microsatellite markers will facilitate the mapping of genes and QTLs which are of economic importance in barley and support studies of genetic diversity, pedigree analysis and the display of graphical genotypes (Russell *et al.* 1997a; Russell *et al.* 2000; Macaulay *et al.* 2001; Koebner *et al.* 2003; Sjakste *et al.* 2003). In this study, out of the 109 polymorphic primer pairs, only four amplified more than one locus. These results are in contrast with about 20% in bread wheat (Röder *et al.* 1998; Varshney *et al.* 2000; Gupta *et al.* 2002) and 11% of the markers that detected more than one locus in barley (Ramsay *et al.* 2000). In this study, all microsatellite markers were derived from genomic library based on hybridization with only dinucleotide repeats $(GA)_n$ and $(GT)_n$. More microsatellites could available using hybridization with trinucleotide or tetranucleotide motifs in the future.

Fig. 4.1 Linkage map of barley based on the data from the Steptoe/Morex cross (Kleinhofs et al. 1993) left-hand and the Igri/Franka cross (Graner et al. 1991) right-hand. The new microsatellite loci mapped in this study are shown in boldface type, while eight loci mapped with a LOD score < 3.0 are indicated with an asterisk. The SSRs previously published in Ramsay et al. (2000) are underlined. The centromeres are indicated based on a barley map published in http://barleygenomics.wsu.edu and Künzel et al. (2000).

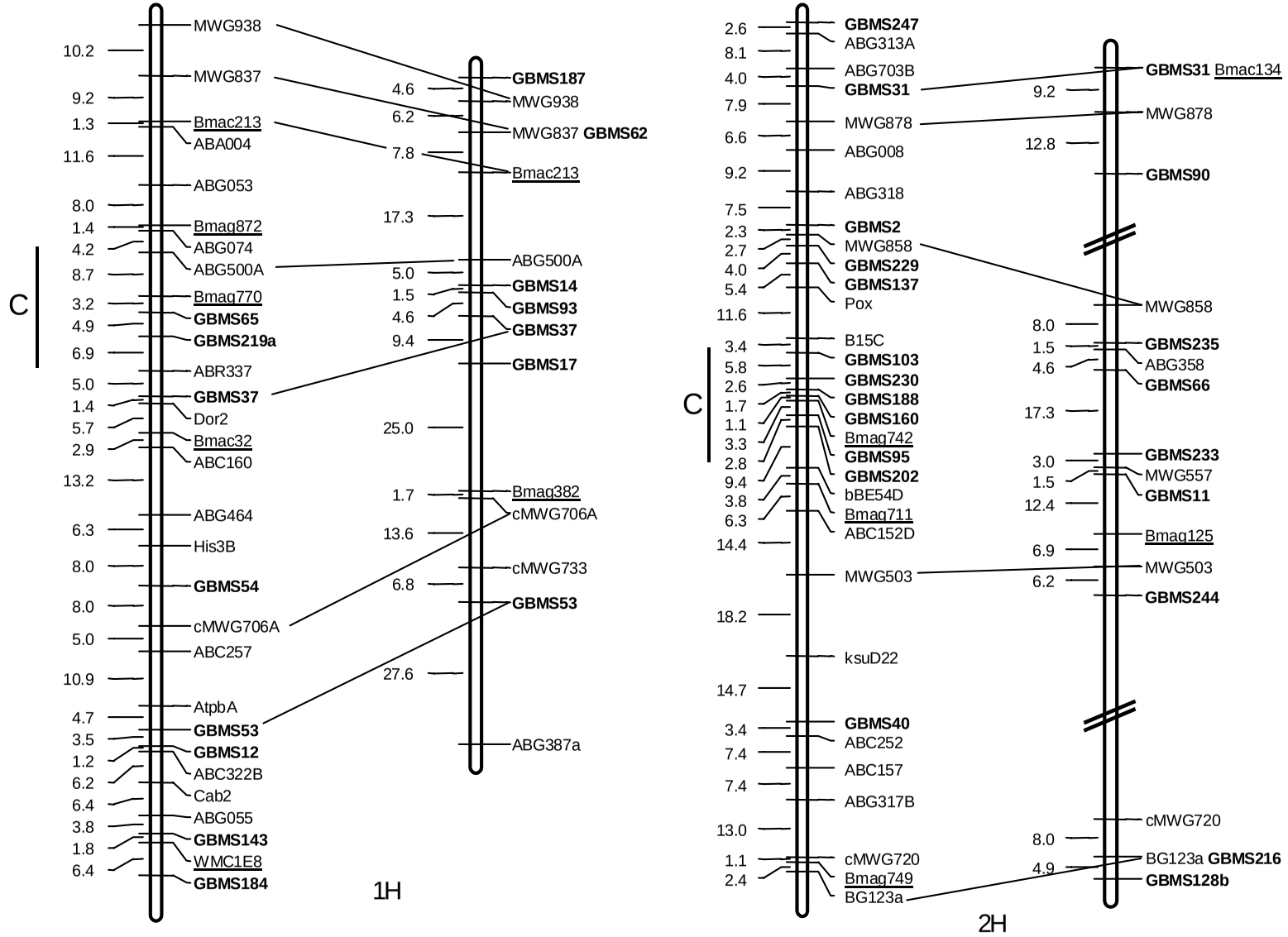
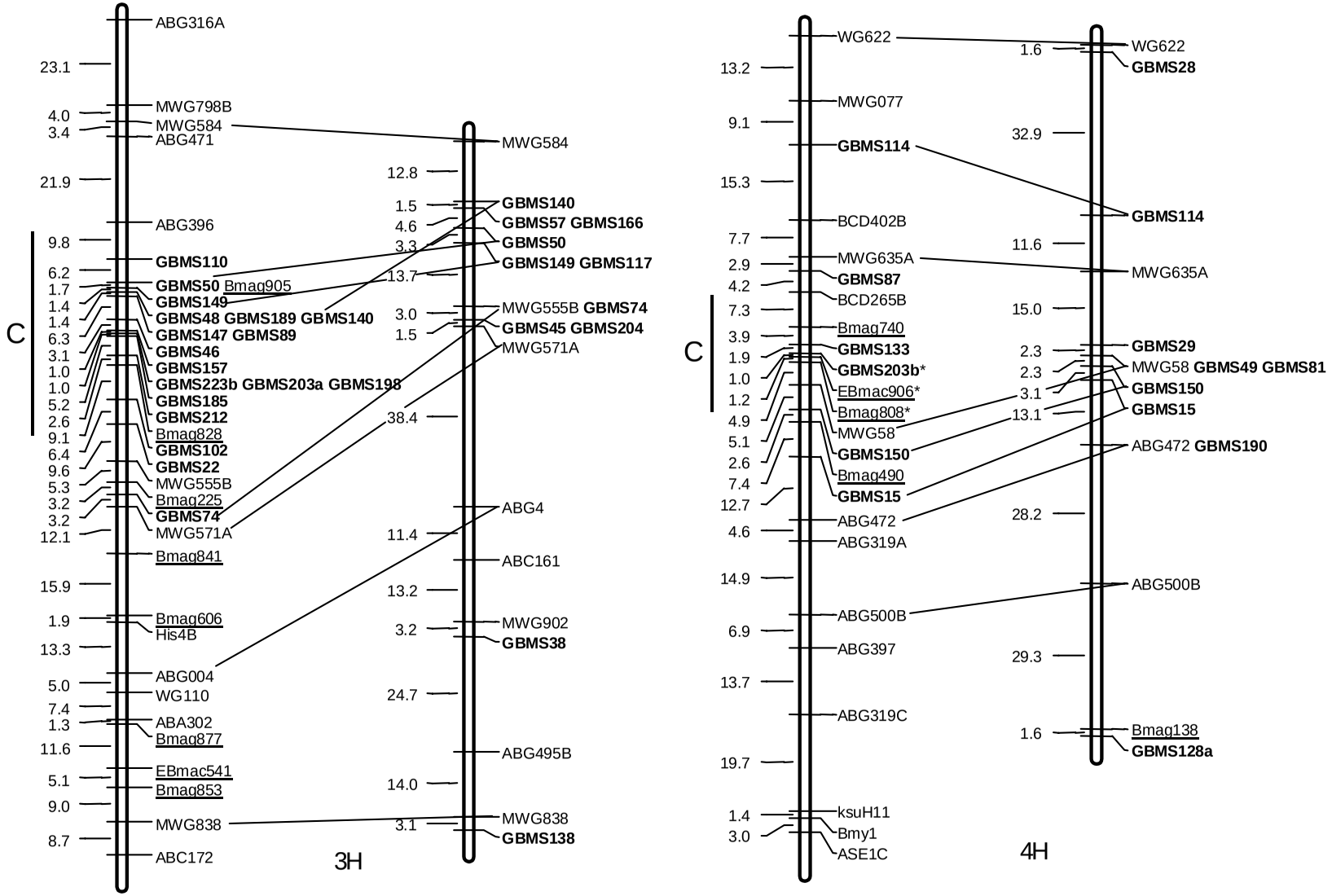

C
MWG938
10.2
MWG837
9.2
1.3
Bmac213
ABA004
11.6
ABG053
8.0
1.4
Bmag872
4.2
ABG074
8.7
ABG500A
3.2
Bmag770
4.9
GBMS65
6.9
GBMS219a
ABR337
5.0
1.4
GBMS37
Dor2
5.7
Bmac32
2.9
ABC160
13.2
ABG464
6.3
His3B
8.0
GBMS54
8.0
cMWG706A
5.0
ABC257
10.9
AtpbA
4.7
3.5
GBMS53
GBMS12
1.2
ABC322B
6.2
Cab2
6.4
ABG055
3.8
GBMS143
1.8
WMC1E8
6.4
GBMS184
1H
GBMS187
4.6
MWG938
6.2
MWG837 GBMS62
7.8
Bmac213
17.3
ABG500A
5.0
GBMS14
1.5
GBMS93
4.6
GBMS37
9.4
GBMS17
25.0
Bmag382
1.7
cMWG706A
13.6
cMWG733
6.8
GBMS53
27.6
ABG387a
C
2.6
GBMS247
ABG313A
8.1
ABG703B
4.0
GBMS31
7.9
MWG878
6.6
ABG008
9.2
ABG318
7.5
2.3
GBMS2
2.7
MWG858
4.0
GBMS229
5.4
GBMS137
Pox
11.6
B15C
3.4
GBMS103
5.8
GBMS230
2.6
GBMS188
1.7
GBMS160
1.1
Bmag742
3.3
GBMS95
2.8
GBMS202
9.4
bBE54D
3.8
Bmag711
6.3
ABC152D
14.4
MWG503
18.2
ksuD22
14.7
3.4
GBMS40
ABC252
7.4
ABC157
7.4
ABG317B
13.0
1.1
cMWG720
2.4
Bmag749
BG123a
2H
GBMS31 Bmac134
9.2
MWG878
12.8
GBMS90
MWG858
8.0
1.5
GBMS235
4.6
ABG358
GBMS66
17.3
3.0
GBMS233
1.5
MWG557
GBMS11
12.4
Bmag125
6.9
MWG503
6.2
GBMS244
cMWG720
8.0
BG123a GBMS216
4.9
GBMS128b

Fig. 4.1 Linkage map of barley



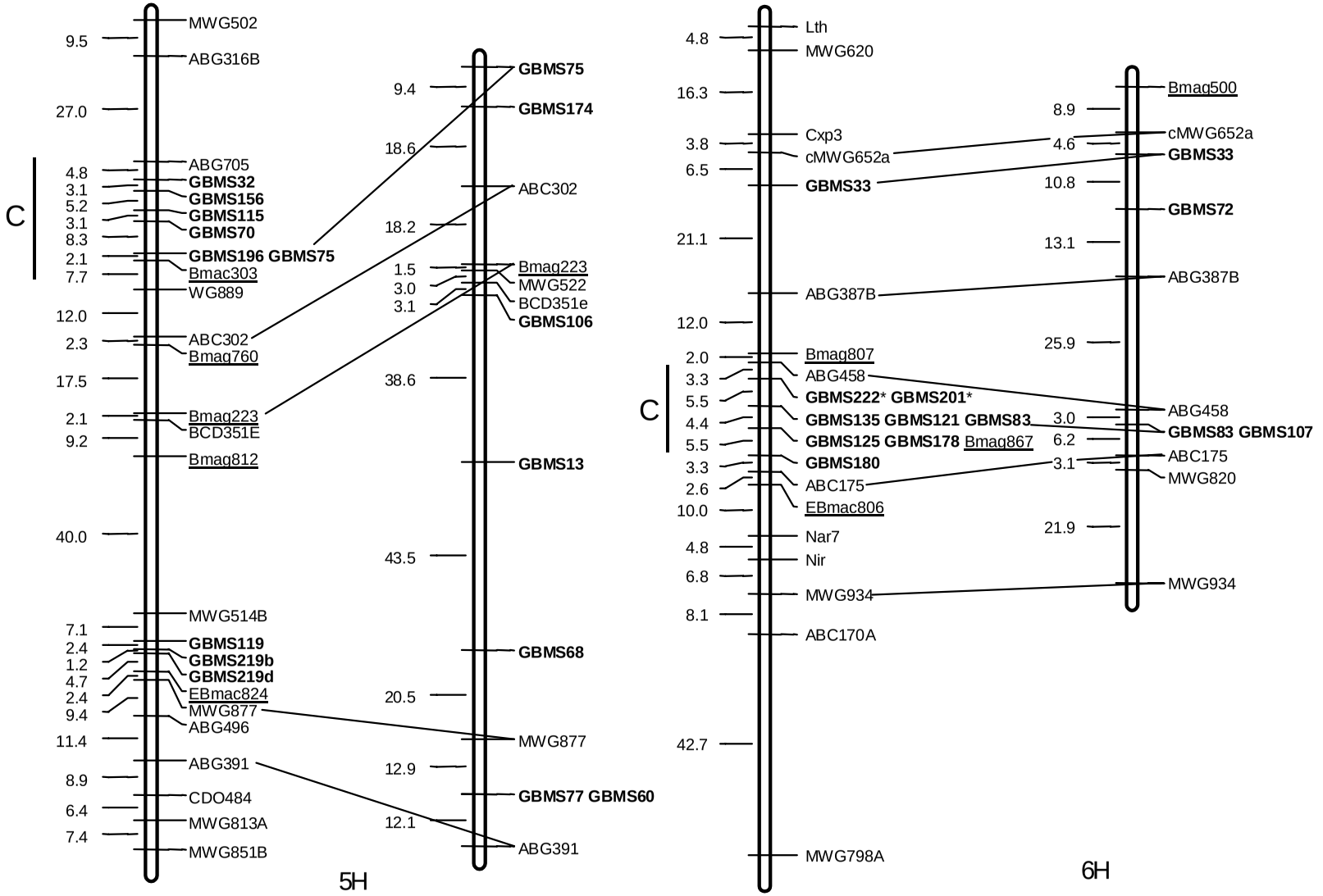
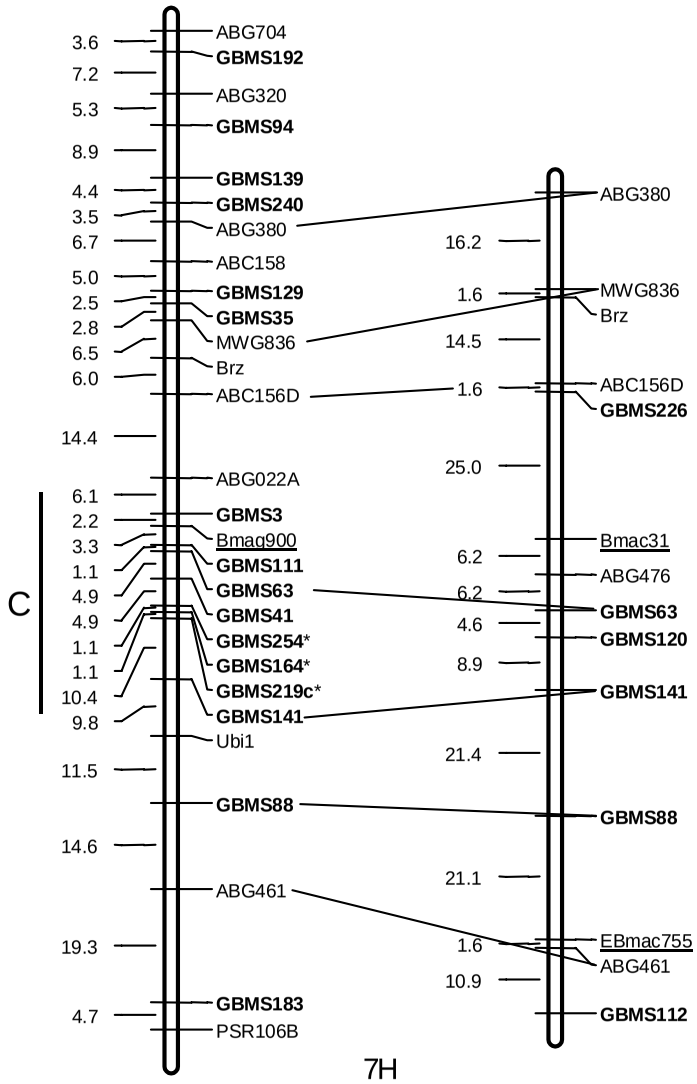

MWG502
9.5
ABG316B
27.0
C
4.8
ABG705
3.1
GBMS32
5.2
GBMS156
3.1
GBMS115
8.3
GBMS70
2.1
GBMS196 GBMS75
7.7
Bmac303
WG889
12.0
2.3
ABC302
Bmag760
17.5
2.1
Bmag223
BCD351E
9.2
Bmag812
40.0
7.1
MWG514B
2.4
GBMS119
1.2
GBMS219b
4.7
GBMS219d
2.4
EBmac824
9.4
MWG877
ABG496
11.4
ABG391
8.9
CDO484
6.4
MWG813A
7.4
MWG851B
5H
9.4
GBMS75
GBMS174
18.6
ABC302
18.2
1.5
Bmag223
3.0
MWG522
3.1
BCD351e
GBMS106
38.6
GBMS13
43.5
GBMS68
20.5
MWG877
12.9
GBMS77 GBMS60
12.1
ABG391
4.8
Lth
MWG620
16.3
3.8
Cxp3
cMWG652a
6.5
GBMS33
21.1
ABG387B
12.0
C
2.0
Bmag807
3.3
ABG458
5.5
GBMS222* GBMS201*
4.4
GBMS135 GBMS121 GBMS83
5.5
GBMS125 GBMS178 Bmag867
3.3
GBMS180
2.6
ABC175
10.0
EBmac806
4.8
Nar7
6.8
Nir
MWG934
8.1
ABC170A
42.7
MWG798A
6H
8.9
Bmag500
4.6
cMWG652a
GBMS33
10.8
GBMS72
13.1
ABG387B
25.9
ABG458
3.0
GBMS83 GBMS107
6.2
ABC175
3.1
MWG820
21.9
MWG934

Fig. 4.1 Linkage map of barley



5 QTL mapping

5.1 Introduction

Advanced backcross QTL analysis has been successfully applied in detecting and transferring QTLs from unadapted germplasm into elite breeding lines for various plant species. Two barley advanced backcross populations were developed and were planted at two locations in three years, separately. The common recurrent parent was a German spring barley cultivar 'Brenda', and the donor parents were two wild species (*Hordeum spontaneum*) HS213 and HS584, respectively. The objective of this study was to evaluate the populations for their agronomic performance and malting quality followed by the combination of genotypic data and phenotypic data of each population, and to identify quantitative traits loci and to generate isogenic lines for interesting traits.

5.2 Mapping QTL in a BC_3-DH population from a cross 'Brenda' × HS213

5.2.1 Materials and methods

5.2.1.1 Population development

The population development was performed by Dr. M. Ganal in Gatersleben with Saatzucht Hadmersleben GmbH. *Hordeum vulgare* sub sp *spontaneum*, 'HS213' was received from Bundesforschungsanstalt für Züchtungsforschung (Aschensleben, Germany) and registered as "Sp.213" in the Genbank of the IPK, and used in two previous studies (Ramsay et al. 2000; Li et al. 2003). It is available under the Gatersleben accession number HOR12530. 'HS213' was crossed as a male parent to 'Brenda'. F_1 plants were grown in greenhouse, and the seven F_1 plants were backcrossed to 'Brenda' (as the female). One hundred one BC_1F_1 plants were obtained and were backcrossed a second time to the 'Brenda' (as the male) to produce BC_2F_1 seeds. 68 BC_2F_1 plants were selected randomly to backcross a third time to the 'Brenda' to generate BC_3F_1 seeds. Two hundred and seventy BC_3DH plants were gained from 39 BC_3F_1 lines with anther culture by Saaten-Union in 1999 (Fig. 5.1). Based on their performances in the field, one hundred and eighty one doubled-haploid (DH) lines were selected for evaluation of agronomic traits.

5.2.1.2 Fine mapping of QTL for heading date

A near isogenic line (G98/65-3/1, selected from the BC_3-DH population) carrying a QTL of interest was chosen as donor parent and crossed to 'Brenda' to generate an F_1. The progeny of 234 F_2 plants and the two parents were planted in greenhouse to investigate the heading date. Phenotypic data combined with genotypic data based on four molecular markers located on chromosome 2HS were used for fine-mapping.

5.2.1.3 Field tests and evaluation of agronomic traits, diseases resistance and malting quality

The progeny and parents were planted in Gatersleben in springs of 2000 and 2001, with four rows and 15 plants each row (3 meter). This population also was grown in plots (5.9m × 1.25m) in Hadmersleben in 2001 and 2002 in collaboration with Dr. F. Heinrichs (Saatzucht Hadmersleben GmbH). Only 81 and 116 plants families were evaluated in Hadmersleben in 2001 and 2002 respectively because of lack of enough seed for all lines. Each DH-line and the

parents were evaluated for seven agronomically important quantitative traits, disease resistance and malting quality (Table 5.1).

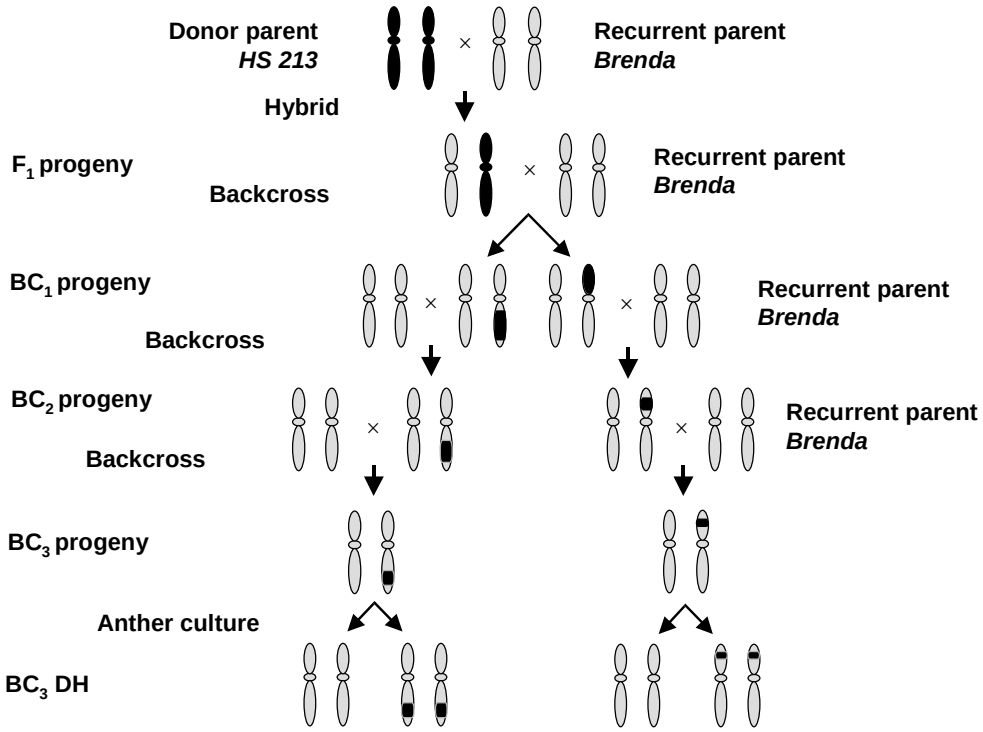


Fig. 5.1 Development of a BC_3-DH population

Table 5.1 A summary of interesting traits investigated at two locations

	Location		Gatersleben				Hadmersleben			
	Year		2000		2001		2001		2002	
	No. of measured lines		113		175		81		110	
	Trait	Abb.	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]
1	Yield (da/ha)	*yld*					×	Plot	×	Plot
2	Heading date (days)	*hd*	×		×		×		×	
3	Plant height (cm)	*ph*	×	3	×	3	×	Average of plot	×	Average of plot
4	Ear length (cm)	*el*	×	6	×	3			×	10
5	Spikelets No. per ear	*sps*	×	6	×	3	×	5	×	10
6	Grains No. per ear	*gps*	×	6	×	3	×	5	×	10
7	1000-grains weight (g)	*tgw*	×	1	×	1	×	1	×	1
8	Spikes No. per plant	*spk*	×	3	×	3				
9	Lodging (1-10)	*Lg*					×	Plot	×	Plot
10	Powdery mildew (1-10)	*Qml*							×	Plot
11	Leaf brown rust (1-10)	*QRph*					×	Plot	×	Plot
12	Net blotch (1-10)	*Qnb*							×	Plot
13	Grain protein (%)	*gp*					×		×	
14	Malt extract (%)	*me*					×		×	
15	Friablity (%)	*fr*					×		×	
16	β-Glucan (mg/100g)								×	

Abb.-Abbreviation; M[1]-measured; No.[2]-Number of measurements for each trait

Yield: Plot yield in Hadmersleben, as the total weight (kg) of all the grains from the plot converted to dt/ha, measured after harvesting with a combine and purifying with a

stationary threshing device. For the four rows planted in Gatersleben in two years, the trait of total yield was not measured.

Heading date: Number of days from sowing to half emergence of the ear

Plant height: Plant height from ground to the tip of the ear (excluding awns) at maturity

Ear length: Length of ear assessed at maturity

Lodging: Visual rating (1–9) of the severity of lodging at harvest

Spikelets No. per ear: Number of spikelets, collected from an ear at maturity

Grains No. per ear: Number of grains, excluding empty or unfilled spikelets from an ear at maturity

1000-grains weight: Weight of thousand grains after harvest

Powdery mildew, leaf brown rust, and net blotch:
Visual rating (1-9) of sensitivity of diseases at maturity in collaboration with cooperating breeders

Malting quality: Data were available made by Saatzucht Hadmersleben GmbH. The analyses were performed at VLB Berlin.

5.2.1.4 Genotyping and linkage analysis

Approximately 400 microsatellite markers from public sources (Liu *et al.* 1996b; Ramsay *et al.* 2000; Li *et al.* 2003) were used to survey the polymorphism between two parents of the population. The 60 polymorphic markers were used to genotype all BC_3-DH plants. The estimated order and distance of the markers was based on the molecular map of barley in two mapping populations 'Steptoe' × 'Morex' and 'Igri' × 'Franka' performed using Mapmaker 3.0b (Lander *et al.* 1987) and Carthagene (Schiex and Gaspin 1997). Segregation ratio of individual markers were statistically determined for each marker locus and deviations from the expected ratio were determined using the chi-square (χ^2) test.

The methods of the DNA extraction and genotyping were according to previous descriptions (Charter 4.2.3). Genomic DNA was extracted from 20-30 g homogenized barley leaf tissue.

5.2.1.5 QTL analysis and statistical analysis

Correlations between traits were analyzed with the Qgene program 3.0 (Nelson 1997). Using SPSS for Windows (SPSS Inc.), One-way ANOVA was performed to test the significances of differences between the genotypes of the population lines and between the years (environments). Single-marker analysis of QTL was used to determine the effect of each

molecular maker on each trait using Qgene 3.0 (Nelson 1997). Interval mapping was performed to identify the location of each QTL on the chromosome.

According to the criteria of Fulton *et al*. (1997a, 2000), a single maker will be identified as a putative QTL, if a significant effect was observed for that single marker/trait combination with $P<0.001$ in only one investigation; significant effects in the same direction were observed for a single/trait combination with $P<0.01$ in two or more investigations; the significant effects in the same direction were observed for a single maker/trait combination with $P<0.1$ in three or more investigations. The percentage of phenotypic change (A%) of each significant QTL at a given maker locus, was calculated as 100(BB-AA)/AA, where AA is the phenotypic mean for individuals homozygous for 'Brenda' alleles at specified markers and BB is the phenotypic mean for individuals homozygous for *Hordeum spontaneum* (Fulton *et al.* 2000). The percent phenotypic variance (%PV) associated with each significant QTL was calculated from the regressions of each maker/phenotype combination in Qgene.

5.2.2 Results

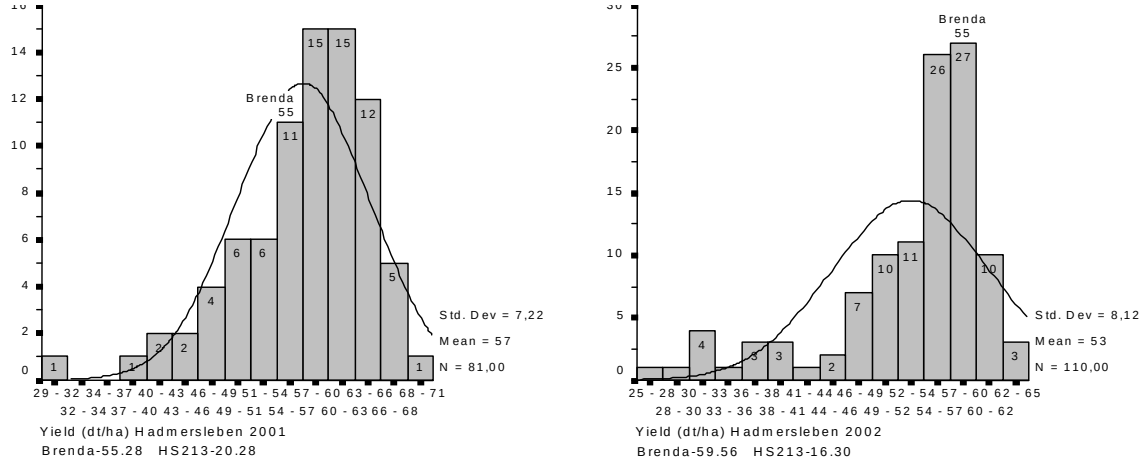
5.2.2.1 Microsatellite polymorphism and marker segregation

Approximately 400 SSR makers were used to survey polymorphism between the recurrent parent 'Brenda' and the donor parent 'HS213', and 15% of them detected polymorphism. Three chromosomes 1H, 3H and 6H showed extensive monomorphism. The lack of microsatellites resulted in two gaps on the long arm of chromosome 2H and 5H, respectively. The observed average allele frequency for HS213 alleles was 4.07%, close to the expected value for a BC_3-DH population of 6.25%. Of the 60 markers used for genotyping the whole BC_3-DH population, 11 (18.3%) deviated significantly ($P<0.05$) from the expected 93.75 : 6.25 allele frequency, two of which showed no transmission of donor alleles at chromosome 2H and 4H. Eight loci were skewed toward 'Brenda' whereas three were skewed toward HS213. Skewing toward the adapted, elite parent can be explained by the selection imposed in the BC_1 and BC_2 generations during population development. Of the 181 lines scored, 110 (61%) were characterized by the presence of the overlapping introgressions from *Hordeum spontaneum*. In the present study, 34 plants containing a single donor segment were found, which can be regarded as defined introgression lines.

5.2.2.2 Distributions of traits

The frequency distributions of phenotypes for yield at Hadmersleben in two years are shown in Figure 5.2. All traits showed approximately normal distributions besides the traits of lodging measured in a combination of Hadmersleben/2001 and leaf brown rust investigated in Hadmersleben/2002. With the enlargement of the number lines surveyed in plots, from 81 in 2001 to 110 in 2002, standard deviation of variances increased. Compared with difference of yield between 'Brenda' and HS213 in 2001, the gap in 2002 expanded. In all cases, the traits for days to heading of two parents showed a consistent result that the heading date of wild species always was earlier than 'Brenda'. Significant differences of plant height of two parents among different combinations (place/year) were observed that might be caused by fertility, climate and the use of chemicals. At Gatersleben in both years, a large number of doubled-haploid lines were found with shorter plant height than the cultivated parent. While the average values for the trait ear length of the two parents were very similar, a broad distribution was observed in the population. The variation of the means of the thousand-grain weight investigated ranged from 38g to 54 g, which would affect the total yield indirectly. In all cases, the thousand-grain weight of the donor parent HS213 was lower than that of 'Brenda' and the doubled-haploid lines also showed a broad phenotypic range. The spike number per plant was only assessed at Gatersleben over two years. It is quite interesting that the lodging evaluation at Hadmersleben in the different years was completely different. More than 90% plants did not lodge in the first year, while most of them were lodged and labeled as slant in the following year, which might be explained the different plant height (76cm and 97cm, respectively) between the two years. While most progeny plants were resistant to powdery mildrew, the sensitivities of this population to two fungal diseases, leaf rust and net

Fig. 5.2 Frequency distribution of phenotypes for yield in the BC_3-DH families
(The data for the other traits not shown)



blotch, were distributed normally and continuously. Protein content and other malting traits were also normally distributed, which showed that alleles from the wild species are involved in quality traits such as protein content, percentage of malting extract and others.

5.2.2.3 Correlations between traits and ANOVA for genotype and environments

For yield and malting traits measured only in one place, correlation coefficients between traits were analyzed separately for each year. As summarized in Table 5.2 (page 52), yield revealed significant positive correlations with spikelet number and grain number per spike, respectively. A significant negative relationship between yield and 1000-grain weight was found in one year. Decreased protein content was associated with increased yield. Days to heading was positively correlated with spikelet number and grain number per ear in both years but not always significantly correlated with the other yield traits. A negative correlation between heading date and plant height at highly singnificant level was followed by a not significant correlation in next year. Both correlations between heading date and lodging were highly significant. However, one was positive and the other was negative. Plant height was positively correlated with lodging in 2001. Ear length also showed significantly positive correlations with the spikelet number and grain number per ear in 2002. The two strongest correlations were observed between spikelet number and grain number per spike in both years (r=0.879 and r=0.922, $P < 0.001$, respectively). Considering the malting traits, protein content showed a positive correlation with friability in 2002, instead of a significant negative one the year before. The result of the ANOVA is presented in Table 5.3. A significant difference was found for yield ($P < 0.05$) among the population lines. ANOVA revealed highly significant environmental effects for all investigated traits.

Table 5.3 The F-value of ANOVA for genotype and environment in the BC_3DH population of the cross 'Brenda' × HS213

Item	Genotype			Environment		
	df	*F*-value	*P*-value	*df*	*F*-value	*P*-value
Heading date	139	0.568	0.994	1	401.923	0.000
Plant height	139	0.645	0.974	1	293.701	0.000
Spikelet No.	139	1.039	0.451	1	34.622	0.000
Grain No.	139	1.205	0.232	1	24.894	0.000
1000 grains weight	140	0.520	0.998	1	280.090	0.000
Yield	140	1.706	0.017	1	13.644	0.000
Leadf brown rustR.	140	0.449	1.000	1	265.499	0.000
Lodging	140	0.642	0.975	1	107.142	0.000
Protein content %	140	0.846	0.773	1	124.366	0.000
Friability	140	0.608	0.986	1	139.467	0.000
Extract %	140	1.440	0.075	1	9.091	0.003

5.2.2.4 QTL detection

Total yield and its component traits and three malting quality traits were evaluated on BC_3DH plots in Hadmersleben in 2001 and 2002. Six main agricultural traits were measured in Gatersleben for two years. Putative QTLs for each trait by single-marker analysis were listed in Table 5.4, according to the criteria by Tanksley et al. (1996) and Fulton et al. (1997a, 2000). The chromosomal location of QTLs is depicted in Fig. 5.3.

5.2.2.4.1 QTLs for yield

Yield was affected by five significant QTLs, which explained from 13.2% to 22.3% of the phenotypic variance. For all of these QTLs, the 'Brenda' alleles increased total yield. For *yld2.1* and *yld2.*2 on chromosome 2H, significant effects on yield were observed in both years. In this case, no HS213 alleles increasing yield could be found.

5.2.2.4.2 QTLs for heading date

Heading date was evaluated at two locations. Eight putative QTLs were detected on all chromosomes except chromosomes 3H and 7H. For all of them, the HS213 alleles reduced the number of days to heading. The most significant QTL associated with heading date was *hd2.2*, which explained 22.4% of the phenotypic variance.

5.2.2.4.3 QTLs for plant height and lodging

Two putative QTLs were detected which significantly affected plant height. A favorable effect of the donor allele, *ph2.1*, which caused a decrease of the plant height, was observed. Lodging was investigated only in one location for two years. Two QTLs associated with lodging on different chromosomes were found in different years. *Lg5.1*, which produced lodging, mapped to the same region of chromosome 5H as *ph5.1*, a QTL for which the HS213 allele increased plant height.

5.2.2.4.4 QTLs for ear length

Four QTLs were located for ear length. All QTLs explained more than 10% of the phenotypic variance. Three of the HS213 alleles caused a decrease in ear length. However, for *el7.1* on chromosome 7H, a significant increase in ear length was detected.

Table 5.2 Partial correlation matrix of the traits analyzed in Hadmersleben.

	Yield	Heading date	Plant height	Ear length	Spikelet No. per ear	Grain No. per ear	1000-grain weight	Resistance to leaf brown rust	Logding	Friability	Protein content
Heading date	0.520*** 0.136										
Plant heigth	0.138 -0.176	-0.547*** -0.137									
Ear length	- 0.054	- 0.263**	- 0.226*								
Spikelet No. per ear	0.354*** 0.207*	0.500*** 0.499***	0.021 0.123	- 0.639***							
Grain No. per ear	0.339** 0.364***	0.518*** 0.601***	0.049 -0.027	- 0.492***	0.922*** 0.879***						
1000-grains weight	-0.416*** 0.074	-0.261* -0.172	0.106 0.125	- 0.096	-0.242* -0.042	-0.247* -0.011					
Resistance to Leaf brown rust	-0.069 0.031	0.119 -0.104	-0.042 0.020	- -0.174	0.067 -0.176	0.150 -0.098	0.113 0.186				
Lodging	-0.242* 0.160	-0.643*** 0.377***	0.762*** -0.048	- 0.016	-0.285* 0.221*	-0.280* 0.277**	0.292** 0.180	-0.035 0.229*			
Friability	0.163 -0.507***	0.308** -0.338***	-0.385*** 0.129	- -0.065	0.165 -0.264**	0.188 -0.346***	0.031 0.109	0.239* 0.074	-0.381*** -0.096		
Protein content	-0.460*** -0.554***	-0.134 -0.463***	0.034 0.199*	- -0.133	-0.219 -0.341***	-0.235* -0.417***	0.208 -0.075	0.024 0.121	0.214 -0.323**	-0.561*** 0.700***	
Extract	0.003 0.473***	-0.035 0.335***	0.083 -0.172	- 0.140	-0.111 0.249*	-0.030 0.283*	-0.009 0.162	-0.028 -0.149	0.069 0.117	0.215 -0.585***	-0.482 -0.816

Hadmersleben 2001
Hadmersleben 2002

Significance levels: *P<0.05, **P<0.01, ***P<0.001.
The upper value of each column is the correlation coefficient gained in 2001, and the lower is gained in 2002.

5.2.2.4.5 QTLs for spikelet number and grain number per ear

Seven QTLs were found for spikelet number per ear. These single QTLs explained 11.2%-36.8% of the phenotypic variance with LODs from 2.06 to 8.08. Of them, five identified regions which were also associated with QTLs for grain number per spike. This result was consistent with the high correlation coefficients between these two traits. *Sps2.1* had the overall greatest and most-consistent effects across the four investigations on spikelet number per spike.

5.2.2.4.6 QTLs for thousand-grain weight

In three investigations, only one putative QTL was detected to be associated with thousand-grains weight. Correlation analysis carried out showed a negative relationship between yield and thousand-grain weight (see pervious section, 5.2.2.3). This QTL, *tgw2.1*, from the donor parent, reduced the thousand-grain weight around 10%.

5.2.2.4.7 QTLs for resistance to leaf brown rust

Resistance to leaf brown rust was measured in Hadmersleben and only one significant QTL was observed. The HS213 allele for *Rph5.1* increased the resistance to disease by approximately 20.3% with a LOD of 3.85.

5.2.2.4.8 QTLs for malting quality traits

Three malting traits were evaluated in two years. However, most of the QTLs were effective in the second year. Only one QTL was located for protein content was found on chromosome 2H with a increasing effect of the HS213 allele, accounting for 10.3% of the phenotypic variance. Two putative QTLs were significantly associated with the quality-related trait malt extract. The HS213 alleles for *me1.1* and me2.*1* caused less than 2% reduction in malt extract percentage, respectively. Four QTLs were detected for seed friability. For three, the HS213 allele produced an increase in friability, accounting for 9.81%-29.47% of the phenotypic variance. However, a donor allele, mapped to chromosome 7H, caused a 19% diminution in the seed friability.

Table 5.4 Putative QTL detected in a DH-BC_3 population ('Brenda' × HS213) by single marker analysis and interval mapping

Trait	QTL	Marker	Source	2000 G	2001 G	2001 H	2002 H	LOD	A%	PV%
Yield	*yld1.1*	Bmac90	Brenda	nd	nd	*	****	5.97	-28.99	22.30
	yld2.1	GBMS229	Brenda	nd	nd	****	**	3.76	-35.25	19.26
	yld2.2	Bmag692	Brenda	nd	nd	***	****	4.14	-26.70	16.31
	yld3.1	Ebmag705	Brenda	nd	nd	*	***	3.24	-15.34	13.24
	yld6.1	Ebmac674	Brenda	nd	nd	*	****	4.37	-20.38	16.71
Heading date	*hd1.1*	GBMS219a	Brenda	ns	****	ns	ns	4.39	-15.03	11.10
	hd1.2	Bmac90	Brenda	*	*	***	**	2.42	-8.69	12.99
	hd2.1	HVM36	Brenda	*	ns	**	*	1.58	-3.25	8.58
	hd2.2	GBMS229	Brenda	***	ns	****	***	4.46	-8.14	22.40
	hd2.3	GBMS230	Brenda	**	ns	*	****	3.82	-4.42	15.31
	hd4.1	Bmag490	Brenda	ns	***	*	ns	3.07	-10.41	7.90
	hd5.1	Ebmac684	Brenda	**	ns	*	***	2.80	-5.54	11.87
	hd6.1	Ebmac674	Brenda	*	*	*	ns	0.65	-5.74	2.68
Plant height	*ph2.1*	Bmag692	Brenda	ns	*	*	*	1.06	-27.60	5.84
	ph5.1	Bmac303	HS213	ns	ns	***	ns	3.05	23.60	15.94
Lodging	*lg2.1*	HVM54	HS213	nd	nd	ns	****	4.02	84.21	16.01
	lg5.1	Bmac303	HS213	nd	nd	***	ns	3.40	207.69	17.57
Ear length	*el2.1*	GBMS229	Brenda	ns	ns	nd	***	3.29	-11.54	12.97
	el2.2	GBMS230	Brenda	**	ns	nd	***	2.51	-9.30	10.34
	el5.1	Ebmac684	Brenda	**	ns	nd	****	6.24	-20.18	24.34
	el7.1	GBMS111	HS213	***	ns	nd	ns	2.90	13.8	11.42
Spikelet No. per ear	*sps1.1*	Bmac90	Brenda	*	ns	***	*	2.61	-34.41	13.94
	sps2.1	GBMS229	Brenda	***	*	****	*	8.08	-39.59	36.83
	sps2.2	GBMS230	Brenda	*	ns	***	**	2.68	-20.35	14.30
	sps2.3	Bmag692	Brenda	*	ns	****	ns	4.60	-44.17	23.02
	sps5.1	Ebmac684	Brenda	*	ns	***	**	3.07	-26.60	16.98
	sps7.1	Ebmac603	Brenda	**	ns	**	ns	2.06	-14.07	11.16
	sps7.2	GBMS35	Brenda	*	*	***	ns	2.74	-24.88	14.57
Grain No. per ear	*gps2.1*	GBMS229	Brenda	*	ns	****	*	8.77	-41.10	39.25
	gps2.2	Bmag692	Brenda	ns	ns	****	ns	5.90	-49.42	28.51
	gps5.1	Ebmac684	Brenda	ns	ns	***	ns	2.81	-25.53	15.64
	gps7.1	Ebmac603	Brenda	ns	ns	***	ns	2.60	-15.80	13.92
	gps7.2	GBMS35	Brenda	ns	ns	****	ns	3.92	-29.43	20.20
1000-grain weight	*tgw2.1*	GBMS216	Brenda	**	nd	**	*	2.20	-10.64	8.80
Leaf brown rust R.	*Rph5.1*	Bmag223	HS213	nd	nd	****	ns	3.85	53.31	20.33
Protein content	*pc2.1*	GBMS230	HS213	nd	nd	ns	***	2.53	10.71	10.30
Malt extract	*me1.1*	Bmac90	Brenda	nd	nd	ns	****	3.75	-1.83	14.67
	me2.1	GBMS230	Brenda	nd	nd	ns	***	2.48	-1.59	10.13
Friability	*fr1.1*	Bmac90	HS213	nd	nd	ns	****	8.26	41.57	29.47
	fr2.1	GBMS229	HS213	nd	nd	ns	***	2.47	27.62	9.81
	fr5.1	GBMS77	HS213	nd	nd	ns	***	2.64	36.42	10.47
	fr7.1	Bmag120	Brenda	nd	nd	***	ns	3.41	-19.01	18.92

Significance levels: * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$
nd: no data; ns: not significant.
G = Gatersleben, H = Hadmersleben.
Regions of the genome were identified as putatively containing a QTL with the method described in the materials and method section.

LOD score, A%, and PV% from the case with the underlined *P*-value
The phenotypic change percentage of (A%) of each significant at a given maker locus, was calculated as 100(BB-AA)/AA, where AA is the phenotypic mean for individuals homozygous for variety alleles at specified markers and BB is the phenotypic mean for individuals homozygous for *Hordeum spontaneum*.
The percent phenotypic variance (%PV) associated with each significant QTL was calculated from the regressions of each maker/phenotype combination based on data from the case with the underlined *P*-value.

5.2.2.5 Mapping of *hd2.2* on the short arm of chromosome 2H

As shown in Table 5.4, in Hadmersleben 2001, one strong putative QTL linked to molecular marker *GBMS229* on chromosome 2HS was detected, which was highly significantly associated with heading date, spikelet number per ear and yield with $P<0.001$. It explained 32.6%, 36.9% and 19.26% of the phenotypic variation, respectively. In later surveys of the following year, this locus was the major source of variation in heading date and was found to be a major factor in all other traits. A near isogenic line *G98/65-3/1*, which carried a donor segment covering this region on the chromosome 2HS was identified by three polymorphic markes-*HVM36, GBMS229* and *GBMS2*. In order to map this QTL *hd2.2* precisely and to describe its effects, a population of 235 F_2 plants was derived from a cross of 'Brenda' × *G98/65-3/1*. Frequency distributions of days to heading in the BC_3-DH population and the F_2 population are shown in Figure 5.4. The phenotypic data originating from the BC_3-DH population indicated that heading date showed a normal distribution at two locations for each year, respectively. Furthermore, the data for the flowering time which was investigated in the F_2 population planted in the greenhouse displayed a distribution of the phenotypic data in a segregation ratio of 3:1, with 88 days as presumed a dividing line. Although number of days to heading of 'Brenda' and HS213 varied due to different sowing time in the four experiments, the flowering time of HS213 was in general earlier than 'Brenda's by two or four days (Table 5.5). In the greenhouse test the difference in flowering time between 'Brenda' and HS213 was 12 days. Table 5.6 shows the segregation ratio of *hd2.2* and analysis of genotypic data of four molecular makers. As expected in a F_2 population, segregation of the four markers followed 1:2:1 ratio. Therefore, the QTL, *hd2.2* detected in a backcross population could be mapped in a F_2 population as a single gene based on its genetic and phenotypic data. As a result of mapping, two markers, *GBMS229* and *GBMS2* were relatively close to *hd2.2*, with a linkage distance of 3.9cM and 2.6cM, respectively (Fig 5.5). In addition, three recombination frequencies were 8.2% between *HVM36* and *GBMS229*, 5.3% between *GBMS229* and *GBMS2*, and 9.4% between *GBMS2* and *GBMS137*, respectively.

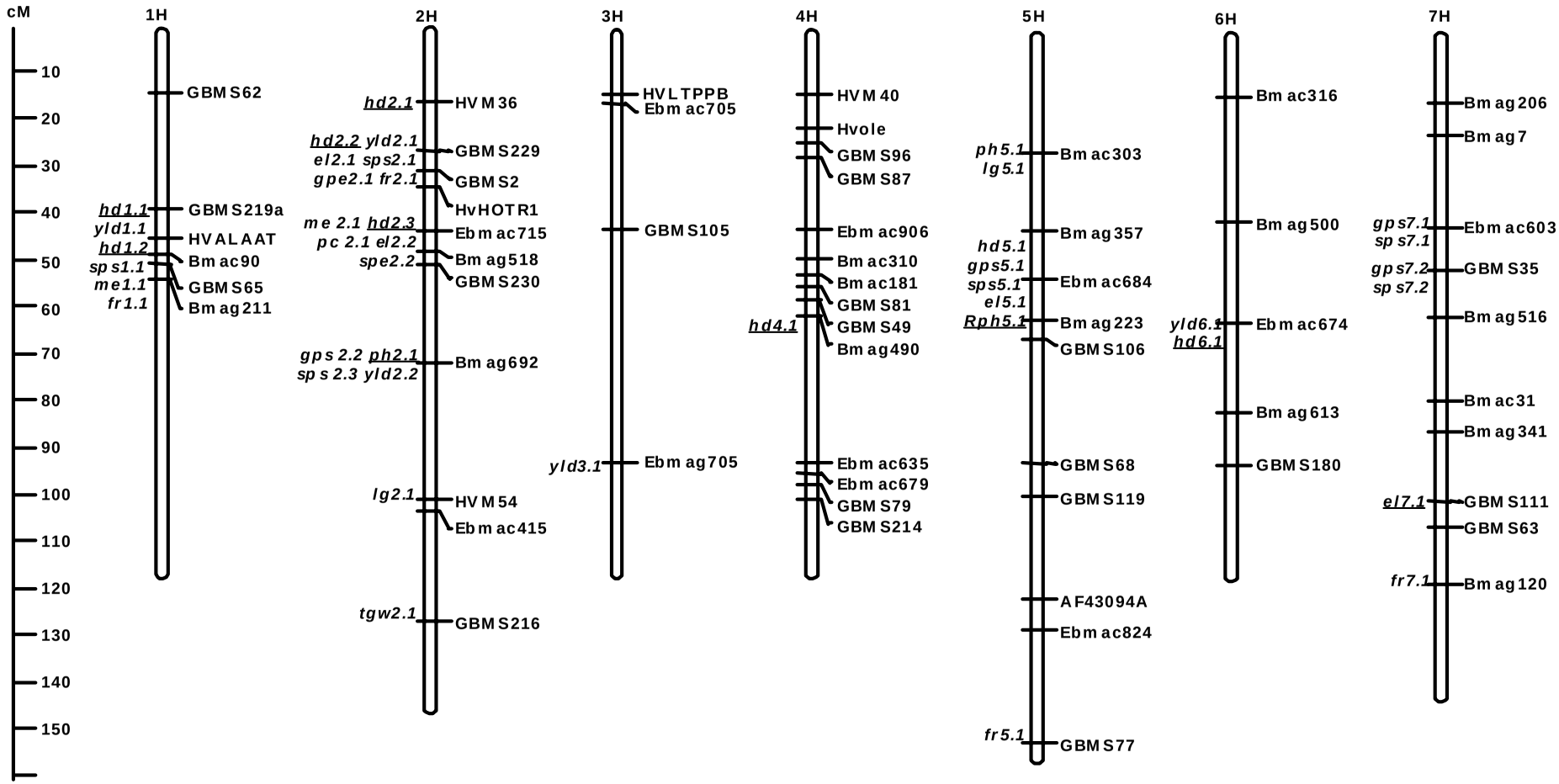


Fig. 5.3 Map locations of the putative QTLs in the Brenda/HS213 population. The order of markers and the distances in cM (Kosambi mapping units) were estimated and based on the barley molecular map (Liu *et al*., 1996; Ramsay *et al*., 2000; Li *et al*., 2003). The centiMorgan scale was given on the left. Locus name were indicated on the right side of the chromosomes. QTLs were designated by the *letter/number combination* to the left of the chromosomes. *Underlined* QTL: the allele from HS213 is favourable for the traits. Abbreviations for traits were listed in Table 5.1.

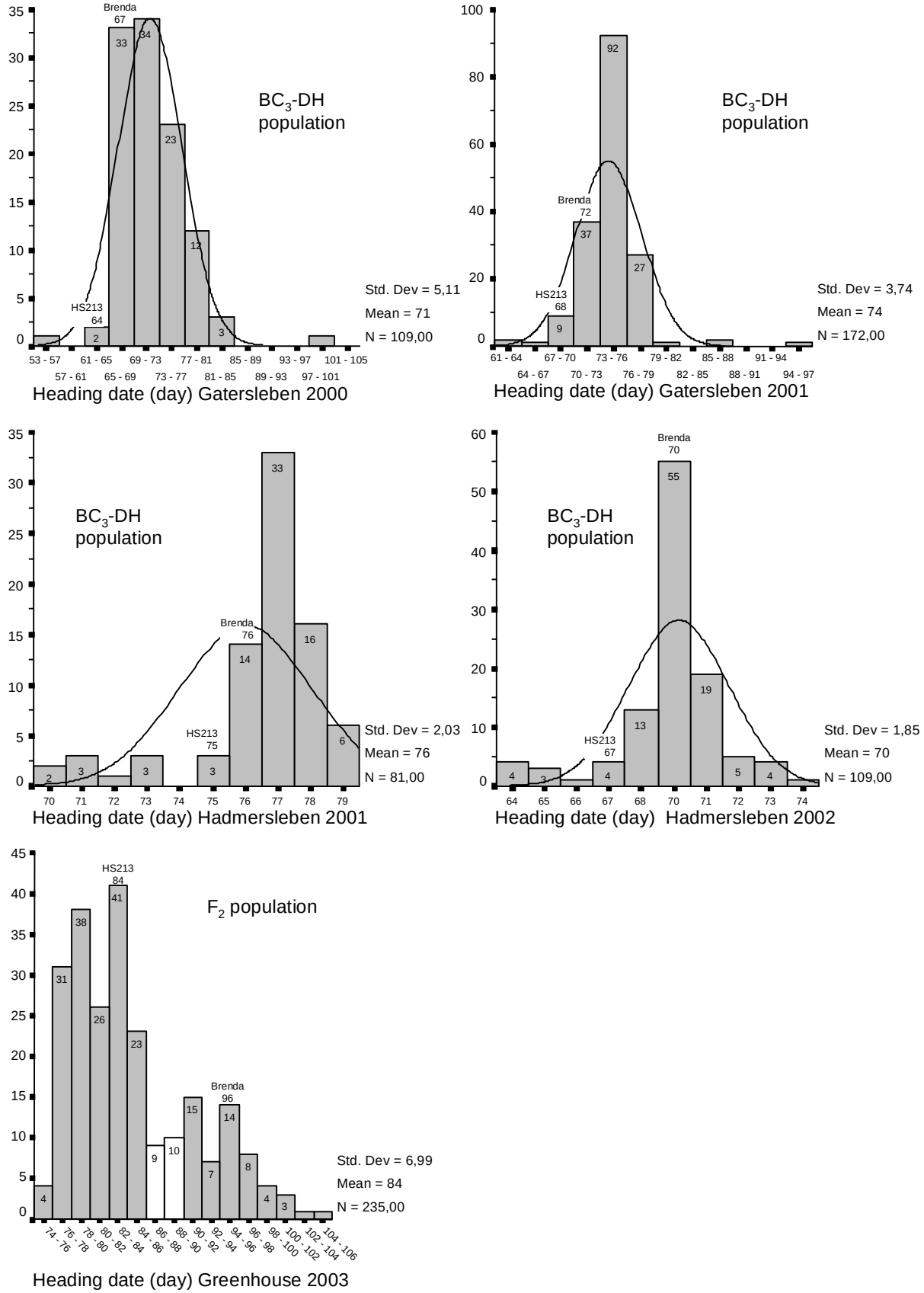


Fig. 5.4 Frequency distribution of phenotypes for heading data in the BC_3-DH families and the F_2 population

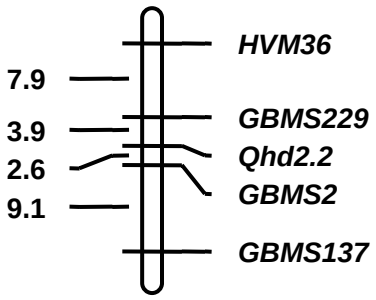


Fig. 5.5 A microsatellite linkage map involving the locus *hd2.2* on the short arm of chromosome 2H. The distances is shown on the left of the chromosome in Kosambi mapping units.

Table 5.5 Phenotypic data for days to heading

Location	Year	Mean (days)	
		Brenda	HS213
Gatersleben	2000	67.00	64.33
	2001	71.83	67.67
Hadmersleben	2001	76.00	74.67
	2002	69.60	67.33
Greenhouse	2003	96.21	84.17

Table 5.6 Segregation analysis for the locus *hd2.2* and microsatellite markers in F_2 population from the cross 'Brenda' × G98/65-3/1

QTL or makers	No. of F_2 plants genotyped	Observed No.			Expected ratio	$\times^2$	P
		X_1X_1	X_1X_2	X_2X_2			
HVM36	218	59	106	53	1:2:1	0.50	0.70-0.80
GBMS229	224	58	108	58	1:2:1	0.29	0.75-0.90
Hd2.2	225	163		52	3 : 1	0.78	0.25-0.50
GBMS2	140	33	77	30	1:2:1	1.53	0.25-0.50
GBMS137	227	63	119	45	1:2:1	3.39	0.10-0.25

5.2.3 Discussion

5.2.3.1 Clustering of QTLs detected in this study

Yield is a comprehensive result of direct characters, for instance, grain weight, grain number per ear and number of spikes per plant, and indirect factors, such as heading date, plant height and environmental factors. Total yield and its components show quantitative variability due to the presence of multiple QTL and environmental variance. Yield components cannot predict yield as well as a measure of yield itself. However, the information based on the measurements of these component characters can provide a better understanding of the reason for the yield differences. Some QTLs were highly genetically correlated and mapped to similar positions. For example, QTLs for spikelets number per ear and grains number per ear always mapped to the same regions, such as on chromosomes 2H, 5H and 7H, while they showed highly significant correlations among different years (Table 5.2, page 52). On chromosome 2H, two QTLs for yield were mapped close to these two traits, resulting in two clusters of QTLs. The clustering of QTLs for other traits was also observed in the centromere

region of chromosome 1H, similar to the report by Pillen *et al.* (2003). Moreover, accumulations of QTLs were found on chromosomes 2H and 5H, respectively. The clustering of QTLs can be explained generally either by a gene with pleiotropic effects or by the occurrence of multilocus clusters in the barley genome (Hayes *et al*. 1993; Oziel *et al*. 1996; Kicherer *et al*. 2000). The marker density of the map also has appreciable effects on QTL detection and resolution, especially in the big gaps on chromosomes 1H and 3H of this study.

5.2.3.2 Comparisons with other QTL studies in barley

Many studies of QTL mapping on yield and quality-related traits were reported by different groups. More than 22 populations were used to detect putative QTLs for yield, agronomic traits, malting-quality and disease resistance etc. in barley (Hayes *et al*. 1993; Backes *et al.* 1995; Bezant *et al.* 1997a and b; Marquez-Cedillo *et al.* 2000; Thomas, 2003). While most of these studies were based on DH, RIL and F_2 populations termed as "classical" QTL analyses, the first AB-QTL analysis was published by Pillen *et al.* (2003) with 45 SSRs. Whereas most classical studies in barley were carried out with RFLPs and AFLPs, in the present study 60 polymorphic microsatellite markers were used to detect the QTLs for yield and its components in the BC_3DH population from the cross 'Brenda' × HS213. An indirect comparison of QTL effects is possible with common markers in several maps (Li *et al.* 2003; Ramsay *et al.* 2000; Kleinhofs *et al.*1993; Graner *et al.* 1991), especially based on BIN maps of the 'Steptoe' / 'Morex' population. (http://barleygenomics.wsu.edu/databases/databases.html). Some of the observed QTL effects in this study can be related to known genes which were previously mapped as Mendelian factors.

5.2.3.2.1 QTLs for yield

The results of ANOVA showed that environmental factors influenced genotypes for yield and yield components in this study. However, five yield-increasing QTL were mapped on chromosomes 1H, 2H, 3H and 6H. All alleles from 'Brenda' were associated with a positive effect for yield. The QTL, *yld2.1* mapped on a region of chromosome 2HS, which was supposed to be affected strongly by a pleiotropic effect of the photoperiodism gene *Ppd-H1* described by Laurie *et al*. (1994, 1995). The QTL, *yld3.1*, mapped on chromosome 3HL near to the region of the *denso* locus, which codes for a dwarfing gene that affects almost all agronomic traits studied by Thomas *et al.* (1991) and Barua *et al.* (1993). Thomas *et al.* (1995) suggested that the *denso* gene was probably very tightly linked to a QTL controlling yield and that the associated QTL depends upon the genetic background. With the Steptoe × Morex

cross, two studies (Hayes *et al.* 1993 and Mather *et al.* 1997) indicated that the number of QTLs influencing yield is depending on the environment.

5.2.3.2.2 QTLs for heading date

Eight putative QTLs for flowering time were mapped on chromosomes 1H, 2H, 4H, 5H and 6H, respectively. All alleles from the wild species reduced the days to heading. Kandemir *et al.* (2000a) reported that the QTL-2S region was associated with flowering time and plant height. In their study, this region was not verified to be identical with the photoperiodism gene, *Ppd-H1* because of missing common markers. This major photoperiod response locus which causes early flowering under long daylength was mapped on the short arm of chromosome 2H, linked to a proximal RFLP marker *XMWG858* with 1cM (Laurie *et al.* 1994). In this study, for heading date, a QTL, *hd2.2*, was mapped on chromosome 2HS linked to *GBMS229* and *GBMS2*. This QTL is presumably the *Ppd-H1* gene. Similar genes were found near the junction between rice 7 and rice 4 linkage segments, and in maize by comparative mapping of cereals (Dunford *et al*. 2002). In this study, the same RFLP markers were mapped in maize to identify regions that might contain *Ppd-H1* orthologs. By producing an F_2-mapping population from a nearly isogenic line containing an introgression at chromosome 2HS that was crossed with 'Brenda', it was possible to demonstrate that QTL *hd2.2* can be traced to a single Mendelian gene which is most probably identical to *Ppd-H1* (see chapter 5.2.2.5). These results demonstrate that nearly isogenic lines containing favorable allele can accelerate the identification and isolation of QTLs as single genes. The other major gene controlling flowering time *Ppd-H2* was located on chromosome 1HL by Laurie *et al*. (1995) while in this study a QTL for heading date was detected around the centromere, which was associated with the same trait in the report by Pillen *et al*. (2003). The QTL on chromosome 5H, *hd5.1*, is located in a similar position as QTL for heading date reported by Thomas *et al.* (1995) and Marquez-Cedillo *et al*. (2001).

5.2.3.2.3 QTLs for plant height and lodging

The correlation between plant height and lodging was significant in 2001, whereas it was not significant in the next year. Only two putative QTLs were identified for these two traits, respectively. One QTL located on chromosome 5HS was associated with increased plant height and lodging. Spanner *et al.* (1999) confirmed the presence of a QTL found by Tinker *et al.* (1996b) affecting plant height and lodging severity at similar location on chromosome 5HS in a two-rowed DH population. Another QTL at locus *HVM54* on chromosome 2H, *lg2.1*

mapped to the same interval as the QTL for plant height *ph2.1* at the neighboring marker *Bmag692*. It represents possibly the same locus controlling height as previously reported by Teulat *et al.* (2001).

5.2.3.2.4 QTLs for ear length and spike number per plant

In this study, four QTLs for ear length were detected. Two mapped on chromosome 2H together with the QTLs for yield. One QTL, *el7.1*, was unlinked to the other traits and found on chromosome 7H. The HS213 allele at this locus increased the ear length. No QTLs were detected for the spike number per plant in this population.

5.2.3.2.5 QTLs for spikelet number per ear and grain number per ear

In the present study, seven and five QTLs for spikelet number and grain number per ear were mapped on chromosomes 1H, 2H, 5H and 7H, respectively. Four QTLs for spikelet number per ear, *sps2*.1, *sps2*.3, *sps5.1*, and *sps7.1* were mapped to similar positions as four QTLs for grain number per ear. The locations of QTLs on chromosomes 1H and 2H for spikelet number and grain number coincided with the QTL for yield. No alleles from wild species HS213 were associated with increasing effects on these two traits. Two QTLs, *sps2.1* and *grs2.1* were located close to the locus *Ppd-H1* on 2HS and are possibly caused by its pleiotropic effects. Kjaer and Jensen (1996) also indicated that QTLs for grain number and thousand-grain weight were located near or at locus *v* on chromosome 2HL due to pleiotropic effects of locus *v*. In the present study, QTL with large effects for yield, plant height, spikelet number and grain number were found near *Bamg692*, which is also near or at locus *v* on chromosome 2H. It is likely that these QTLs are due to the same cause assumed by Kjaer and Jensen (1996). Only one QTL for grains number per ear mapped on chromosome 1HS was reported by Pillen *et al.* (2003). Compared with the positions of the QTL mapped by Bezant *et al.* (1997a) and Teulat *et al.* (2001), no QTL could be identified in this study. This could be explained by the fact that those QTLs were found in different crosses. Based on the S/M population, Kandemir *et al.* (2000a) reported only one QTL for spike density on chromosome 3H, sharing the same position as the head shattering QTL *Hst-3*. During population development of the ‘Brenda’ / HS213 population a selection was imposed against brittle rhachis, therefore head shattering was not observed in the doubled haploid lines.

5.2.3.2.6 QTLs for thousand grain weight

In this study, only one putative QTL for thousand-grain weight, *tgw2.1*, was mapped on the long arm of chromosome 2H. It was verified in three cases although with low LOD scores, of less than 3.0. The closest QTL published to *tgw2.1* was mapped on BIN14 group on the bottom of chromosome 2H by Bezant *et al*. (1997a). The HS213 allele decreased the thousand-grain weight for this QTL.

5.2.3.2.7 QTLs for resistance to leaf brown rust

A putative QTL, *Rph5.1*, associated with resistance to leaf brown rust was detected at Hadmersleben in 2001. It was mapped in an interval between *Ebmac684* and *Bmag223* on chromosome 5HL. The resistance comes from HS213. *Rph2* and *Rph9* were mapped on chromosome 5HS and 5HL, repectively (Borovkova *et al.* 1997, 1998). *Rph2* was placed in BIN 7 while the position of *Rph9* belonged to BIN 11. Thus, *Rph5.1* is a new locus which is different from these two genes.

5.2.3.2.8 QTLs for malting quality---protein content

Only one QTL for protein content was located on chromosome 2HL in the present study. The 'HS213'allele caused an increase in protein content. This QTL coincides with QTLs for yield, heading date, ear length, spikelets number per ear and percentage of malt extract. For malting quality and percentage of kernel plumpness, coincident QTLs were detected at a close position by Marquez-Cedillo *et al*. (2000) and Hoffman *et al*. (2002), respectively. No grain protein effects were found in the segment of chromosome 1HS at the hordein locus because of a gap of makers. About 45% of the protein needs to be solubilized during malting and mashing. Too strong or weak protein solubilization is reported to result in beers with poor foaming characteristics and beer haze precipitates, respectively (Hayes *et al.* 2003). Therefore, loci controlling protein content are primary genetic factors for malting quality in barley.

5.2.3.2.9 QTLs for malting quality--- percentage of malt extract

Malt extract percentage QTLs were detected on chromosomes 1H and 2H in the present study. For both QTLs, the recurrent parent contributed the favourable alleles increasing the percentage of malt extract. They coincided with the clusters of QTLs determining multiple traits, respectively. The QTL for malt extract on chromosome 1HL coincided with a QTL for friability. The other QTL which was mapped to chromosome 2H, corresponding to the QTL for protein content. The detection of the QTL on chromosome 1HL confirmed the discovery

of a QTL for malt extraction at a near by position in two-rowed and six-rowed subpopulations based on results by SIM analysis (Marquez-Cedillo *et al*. 2000).

5.2.3.2.10 QTLs for malting quality---grain friability

A high quality malt requires an appropriate degree of friability to allow the right amount of hydrolytic enzymes and metabolites to be readily solublized during the malt mashing process. For friability, few studies on the identification of QTLs were reported. Here, the alleles derived from HS213 were associated with an increase of friability for three of four QTLs mapped to chromosomes 1H, 2H, 5H and 7H. QTLs on chromosomes 1H and 2H coincided not with other QTLs for malt quality but for agronomic characters. The effects of two QTLs for friability at distal positions on the long arms of chromosomes 5H and 7H did not correspond to any other QTLs for malting quality seen elsewhere in the genome.

5.3 Mapping of QTL in a BC_3 population from a cross 'Brenda' × HS584

5.3.1 Materials and methods

5.3.1.1 Population development

The population development was carried out by Dr. M. Ganal in collaboration with Saatzucht Hadmersleben GbmH. *Hordeum vulgare* sub sp *spontaneum*, 'HS584' was obtained from the Gatersleben Genbank. It was received from the Bundesforschungsanstalt für Züchtungsforschung (Aschersleben, Germany) and registered as "Sp.584" in the Genbank of the IPK, and used in two previous studies (Ramsay et al. 2000; Li et al. 2003). It is available under the Gatersleben accession number HOR12560. HS584 (*Hordeum spontaneum*) was crossed as the male parent to 'Brenda'. F_1 plants were grown in greenhouse, and the seven F_1 plants were backcrossed to the 'Brenda' (as the male). Forty-six BC_1F_1 plants were obtained and were backcrossed a second time to the 'Brenda' (as the male) to produce 305 BC_2F_1 seeds. Fifty-six BC_2F_1 plants were backcrossed a third time to 'Brenda' to generate 1210 BC_3F_1 seeds. Based on their phenotypic performances, 207 BC_3F_2 families were selected for measurement of agronomic traits.

5.3.1.2 Field trials and evaluation of agronomic traits

The progeny and parents were planted at Gatersleben in springs of 2001, 2002 and 2003, with four rows and 15 plants each row (3 meter). This population also was grown in rows in 2002 and in plots (5.9m × 1.25m) in 2003 at Hadmersleben for measuring agronomic traits. All BC_3F_2 families and parents were evaluated for agronomically important quantitative traits and diseases resistance (Table 5.7).

Harvest: Total weight (g) of all the grains collected from each line. Because same acreage was used to plant each line, the variances of this trait were calculated as yield in QTL analysis. For descriptions of the other traits it is referred to the previous materials and method in chapter 5.2.1.2.

Table 5.7 A summary of agronomic traits investigated in two locations over three years

	Location		Gatersleben						Hadmersleben			
	Year		2001		2002		2003		2002		2003	
	No. of plants		193		196		181		189		181	
	Trait	Abb.	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]
1	Harvest (g)								×	Total weight		

	Location		Gatersleben						Hadmersleben			
	Year		2001		2002		2003		2002		2003	
	No. of plants		193		196		181		189		181	
	Trait	Abb.	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]	M[1]	No.[2]
2	Yield (dt/ha)	*yld*									×	plot
3	Heading date (days)	*hd*	×	half[3]	×	half[3]	×	half[3]	×	half[3]	×	half[3]
4	Plant height (cm)	*ph*	×	3	×	10	×	10	×	10	×	10
5	Ear length (cm)	*el*	×	3	×	10	×	10	×	10	×	10
6	Lodging (1-10)	*Ld*									×	
7	Spikelets No. per ear	*sps*	×	3	×	10	×	10	×	10	×	10
8	Grains No. per ear	*gps*	×	3	×	10	×	10	×	10	×	10
9	1000-grain weight (g)	*Tw*	×		×		×		×			
10	Spike No. per plant	*Sp*	×	30	×	30						
11	Powdery mildew (1-10)	*Qml*							×		×	
12	Leaf brown rust (1-10)	*QRph*							×		×	

Abb.-Abbreviation; M[1]-measured; No.[2]-Number of measurements obtained for each trait; Half[3]- half plot

5.3.1.3 Genotyping and linkage analysis

Genomic DNA was extracted from 20-30 g leaves mixed from six barley plants (BC_3 population) to represent the entire genetic background of each line see chapter 5.2.1.3.

5.3.2 Results

5.3.2.1 Microsatellite polymorphism and marker segregation

Of approximately 400 SSR makers used to survey polymorphism between the recurrent parent ‘Brenda’ and the donor parent ‘HS584’, 187 (46.8%) polymorphic marker were detected. According to their published position and distances displayed between common markers mapped based on three maps (Liu *et al.* 1996b; Ramsay *et al.* 2000; Li *et al.* 2003), a total of 108 microsatellites were selected to genotype all BC_3-F_2 lines. In theory, the percentages of homozygous alleles from the donor parent HS584, heterozygotes from two parents ‘Brenda’ and HS584, and homozygotes from recurrent parent ‘Brenda’ should be 0.2%, 9.0% and 90.8% in a BC_3S_1 population (segregation ratio: AA : AB : BB = 128 : 5888 : 59520). However, the average allele frequency were as follows: 2.73%, 6.16% and 91.11%, respectively. The high proportion of homozygous alleles from HS584 in the entire genetic background may be a result of occasional self-pollination in some lines during the backcrossing steps in the development of this population. In this case, the segregation ratio would be changed depending on the time when the self-pollination occurred. Of the 200 lines scored, 197 (98.5%) were characterized by the presence of the overlapping introgressions

from *Hordeum spontaneum*. In the present study, 17 plants containing a single donor segment were found, which can be regarded as defined introgression lines.

5.3.2.2 Distributions of traits

The BC_3-F_2 population from a cross between 'Brenda' and wild species HS584 was grown for agronomic measurements at two places in serval years, with at least 176 lines. All phenotypic data showed approximately normal distributions except the trait of leaf brown rust resistance assessed at Hadmersleben in 2002 (data not shown). Due to a lack of enough seeds for some lines, the population was planted in several rows instead of plots at Hadmersleben for the first year, therefore, only total weight of harvest grains was available. This could not be compared directly to total yield obtained in the plots. In 2003, the mean of the yield from the recurrent parent 'Brenda' was up to 81.97 dt/ha while the mean of all backcross lines was 75 dt/ha. However, several backcross lines had a transgressive yield compared to 'Brenda'. Based on data obtained over five field trials, the average of days to ear emergence time in the whole population was 74.12 days. Generally, 'Brenda' had longer times to maturity than the wild species with an exception at Gatersleben in 2003, which could be explained by environmental effects. The heading dates obtained at both locations in 2002 were significantly shorter, and might be due to the late sowing time in that year.

Plant height had a broad distribution with some variation in the mean and the absolute height of 'Brenda' in the different trials. However in all cases, the plant height of 'Brenda' was close to the mean of the population and many shorter lines were found among the segregates of the population. For ear length, the mean of the recurrent 'Brenda' was higher than the other parent, while in the population many transgressive lines with longer ear length were observed. The same observations were made for spikelets number per ear and for grains number per ear which both were measured five times. The differences among the means for grains numbers were less than those of spikelet per ear. The trait spike number per plant was evaluated at only one location in different years. The phenotypic data for thousand-grain weight displayed a normal distributions in all cases and the highest mean was 50 g, found at Gatersleben in 2003. The phenotypic data for the leaf brown rust also showed a discrete distribution while a normal distribution with a small deviation was observed for the fungal disease powdery mildew.

5.3.2.3 Correlations between traits and ANOVA analysis

The correlation between most traits assessed in this study was estimated separately for each case. The correlation coefficients among the 10 traits is presented in Table 5.8. No clear

correlations were found between yield and its components for five cases, with the only exception of a significant negative correlation between yield and heading date at Hadmersleben in 2002. In most cases, heading date correlated with ear length, and spikelets number and grains number per ear, respectively. Significant positive correlations among plant height, ear length, spikelets number and grains number per ear were found in all combinations.

Table 5.8 Correlation matrix of the traits in the BC_3 population of the cross 'Brenda' × HS584

	Yield	Heading date	Plant height	Ear length	Spikelets number per ear	Grains number per ear	Spike number per plant	1000-grains weight	Resistance to powdery
hd	nd								
	nd								
	nd								
	-0.322***								
	0.052								
ph	nd	-0.386***							
	nd	-0.204**							
	nd	-0.088							
	0.112	-0.033							
	-0.192*	0.011							
el	nd	-0.063	0.491***						
	nd	0.328***	0.343***						
	nd	0.151*	0.245**						
	-0.165*	0.244**	0.47***						
	-0.025	-0.043	0.518***						
sps	nd	-0.006	0.517***	0.642***					
	nd	0.325***	0.395***	0.771***					
	nd	0.216**	0.395***	0.680***					
	0.031	0.328***	0.373***	0.736***					
	0.043	-0.030	0.365***	0.720***					
gps	nd	-0.006	0.542***	0.643***	0.954***				
	nd	0.322***	0.288***	0.638***	0.702***				
	nd	0.239**	0.404***	0.645***	0.939***				
	0.175*	0.246**	0.364***	0.669***	0.903***				
	0.066	-0.036	0.285***	0.705***	0.919***				
spk	nd	-0.169*	0.249**	0.100	0.169*	0.223**			
	nd	-0.142*	-0.042	-0.296***	-0.2140**	-0.153*			
	nd	nd	nd	nd	nd	nd			
	nd	nd	nd	nd	nd	nd			
	nd	nd	nd	nd	nd	nd			
tgw	nd	-0.183*	0.059	-0.026	0.002	-0.014	-0.014		
	nd	-0.159*	0.070	0.022	0.044	0.076	0.025		
	nd	-0.169*	0.386***	-0.054	0.101	0.110	nd		
	-0.064	-0.389***	0.314***	0.159*	-0.076	-0.035	nd		
	nd	nd	nd	nd	nd	nd	nd		
ml	nd	nd	nd	nd	nd	nd	nd	nd	
	nd	nd	nd	nd	nd	nd	nd	Nd	
	nd	nd	nd	nd	nd	nd	nd	Nd	
	0.058	0.025	0.075	0.087	0.085	0.111	nd	0.03	
	0.063	0.011	ns	-0.064	0.011	0.024	nd	nd	
Rph	nd	nd	nd	nd	nd	nd	nd	nd	nd
	nd	nd	nd	nd	nd	nd	nd	nd	nd
	nd	nd	nd	nd	nd	nd	nd	nd	nd
	0.035	-0.248**	-0.074	-0.107	-0.132	-0.108	nd	0.242**	-0.039
	0.004	0.053	-0.074	0.016	-0.035	0.001	nd	nd	-0.024

2001 Gatersleben
2002 Gatersleben
2003 Gatersleben
2002 Hadmersleben
2003 Hadmersleben

Significance levels: **P*<0.05, ***P*<0.01, ****P*<0.001. nd: no data

The result of the ANOVA is presented in Table 5.9. For each trait no significant difference was found among the population lines whereas ANOVA analysis revealed highly significant differences caused by the environmental effects. The environment showed large influences on all the traits.

Table 5.9 The *F*-value of ANOVA for genotype and environment in the BC_3 population of the cross 'Brenda' × HS584

Item	Genotype			Environment		
	df	*F*-value	*P*-value	*df*	*F*-value	*P*-value
Heading date	199	0.147	1.000	4	2117.766	0.000
Plant height	199	0.473	1.000	4	698.891	0.000
Ear length	199	0.064	1.000	4	22911.531	0.000
Spikelets No.	199	0.226	1.000	4	1821.284	0.000
Grains No.	199	1.045	0.341	4	225.655	0.000
Spike No.	199	0.107	1.000	2	2618.080	0.000
1000 grains weight	199	1.150	0.111	3	262.983	0.000
Powder mildew	194	1.041	0.394	1	6.978	0.009
Leaf brown rust	194	0.097	1.000	1	4083.506	0.000

5.3.2.4 QTL detection

Total yield and its component traits and resistance to diseases were investigated at Hadmersleben in 2002 and 2003. Seven agricultural traits were assessed at Gatersleben in three years. Putative QTLs for each trait by single-marker analysis are listed in Table 5.9, according to a criteria by Tanksley et al. (1996) and Fulton et al. (1997a, 2000). The chromosomal location of QTLs is depicted in Fig.5.4.

5.3.2.4.1 QTLs for yield

Yield was evaluated at Hadmersleben in 2002 and 2003, respectively, however, in plot design only in 2003. Nine QTLs were detected, explaining from 6.07% to 13.96% of the phenotypic variance with a LOD of 2.44 to 6.04. Of all these QTLs, the Brenda alleles increased total yield. The strongest QTL for yield mapped around the centromere on chromosome 6H, which was detected as a main QTL in both years with significant LOD scores over 3.00, at 6.04 and 4.00, respectively. Correspondingly, two QTLs, *yld1.2* and *yld5.1*, which were mapped on long arms of chromosomes 1H and 5H, were also observed as significant increase of production in two years, respectively.

5.3.2.4.2 QTLs for heading date

Heading date was assessed at two locations across three years. Thirteen putative QTLs were detected on all chromosomes except chromosomes 4H. Of them, more than half of the loci

from wild species HS584 reduced the number of days to heading. The most significant QTL associated with heading date was *hd2.1*, with a LOD value at 8.62 by single marker analysis and at 9.74 by interval mapping, which explained 18.67% of the phenotypic variance. The effects of this locus were identified especially at Gatersleben in 2001 and at Hadmersleben in 2003 with the same level of significance, $p < 0.0001$. Of the other three QTLs, *hd1.1*, *hd3.1* and *hd5.1* associated with heading date, *hd3.1* was more reliable than the others since its high significant level could be confirmed at least in three combinations (place/year) with a LOD value higher than 3.00.

5.3.2.4.3 QTLs for plant height and ear length

Twelve putative QTLs were detected which significantly affected plant height. Although most loci from HS584 increased height, favorable effects of two donor alleles, *ph2.1* and *ph2.2* were observed, which caused a decrease in plant height. *Ph2.1*, was a main QTL, which was highly significantly associated with height in three cases ($p < 0.0001$) and explained 22.97% of the variance, whereas phenotypic variances explained by the other minor QTLs were less than 12.0%. Only one QTL, *hd3.3*, was detected in all cases. Plant height of each line was evaluated as a sum of average values of halm length and average values of ear length. Therefore, ear length contributed to plant height. However, among seven putative QTLs identified for the ear length, only two loci, located on chromosome 3H and 7H, had effects on the plant height in the same direction. A QTL, *el2.1* mapped on chromosome 2H with an effect on the ear length and was close to the location of a QTL affecting plant height (Fig. 5.6).

5.3.2.4.4 QTLs for spikelet number and grain number per ear

Eleven QTLs were associated with spikelet number per spike. The single QTLs explained 5.75% - 36.52% of the phenotypic variance with LODs scores from 2.33 to 17.86. Only one QTL from the wild species increased the number of spikelet. The seven identical regions on the barley chromosomes were also found to be associated with QTLs for grain number per spike. This result was consistent with the correlation coefficients between these two traits. It is very interesting to point out that the same region on chromosome 2HS had the greatest and most-consistent effect across five investigations on spikelet number and grain number per spike. The QTLs identified within this interval also controlled aforementioned traits, such as, plant height, ear length and heading date.

5.3.2.4.5 QTLs for thousand-grain weight and spike number per plant

Twelve QTLs influenced significantly thousand-grain weight. The HS584 alleles increased thousand-gain weight at four loci and decreased it at the other eight QTLs. These individual QTL explained 3.77% to 19.92% of the total phenotypic variation. A QTL on chromosome 4H controlling thousand-grain weight was observed at Gatersleben in two continuous years with a highly significant level ($p < 0.0001$). Furthermore, three QTLs on chromosomes 5H, 6H and 7H were significant in four cases, which p values of less than 0.001.

In two investigations at Gatersleben in 2001 and 2002, only three putative QTLs were found to be associated with spike number per plant at low significant level ($p < 0.01$). The magnitude of the phenotypic effect of these single QTLs ranged from 2.59-5.19%.

5.3.2.4.6 QTLs for resistance to leaf brown rust and powdery mildew

Resistances to fungal diseases were evaluated at Hadmersleben in 2002 and in 2003. Only one QTL was associated with resistance to leaf brown rust at very low significant level. Two QTLs showed significant association with resistance to powdery mildew. However, their positive effects on the resistance to disease were from the recurrent parent.

Table 5.10 Putative QTL detected in a BC_3 population ('Brenda' × HS584) by single marker analysis and interval mapping

Trait	QTL	Marker	Source	2001 G	2002 G	2003 G	2002 H	2003 H	LOD	A%	PV%
Yield	*yld1.1*	Bmac90	Brenda	nd	nd	nd	ns	**	2.44	-14.19	6.07
	yld1.2	HVHVA1	Brenda	nd	nd	nd	****	*	5.91	-58.34	13.69
	yld2.1	HVM36	Brenda	nd	nd	nd	ns	***	3.79	-22.54	9.29
	yld 2.2	GBMS229	Brenda	nd	nd	nd	ns	***	3.89	-15.87	9.52
	yld 5.1	EBmac684	Brenda	nd	nd	nd	ns	**	2.23	-15.87	5.58
	yld 5.2	HVM6	Brenda	nd	nd	nd	*	****	4.72	-19.78	11.43
	yld 6.1	EBmac674	Brenda	nd	nd	nd	****	***	6.04	-19.75	13.96
	yld 7.1	GBMS240	Brenda	nd	nd	nd	ns	****	5.72	-17.34	13.68
	yld 7.2	GBMS128b	Brenda	nd	nd	nd	****	ns	5,26	-21.33	12.26
Heading date	*hd1.1*	GBMS62	HS584	ns	ns	ns	***	***	4,20	5.60	10.52
	hd1.2	GBMS14	HS584	*	*	*	**	****	7,36	7.35	17.6
	hd1.3	Bmag718	HS584	ns	ns	***	ns	ns	3.15	4.79	7.78
	hd2.1	GBMS2	Brenda	****	***	**	*	****	8.62	-20.48	18.67
	hd2.2	GBMS216	Brenda	ns	*	**	ns	**	2.65	-4.64	6.71
	hd3.1	Bmag606	Brenda	*	****	*	****	***	5.81	-7.33	12.76
	hd3.2	EBmac541	Brenda	ns	***	ns	***	*	4.27	-4.56	9.87
	hd5.1	GBMS68	HS584	*	*	*	*	****	6.08	7.40	14.7
	hd5.2	EBmac824	HS584	*	*	ns	ns	****	5.85	4.83	14.19
	hd6.1	GBMS222	HS584	ns	*	**	***	****	4.50	2.33	11.11
	hd7.1	Bmag516	Brenda	**	ns	*	ns	*	2.13	-5.37	4.95
	hd7.2	GBMS111	Brenda	**	ns	ns	*	**	2.61	-1.71	6.61
	hd7.3	EBmag757	Brenda	*	ns	ns	*	*	1.78	0.23	4.55
Plant height	*ph1.1*	GBMS184	HS584	***	ns	ns	ns	ns	4.04	19.79	9.2
	ph2.1	GBMS2	Brenda	ns	****	****	****	ns	11.11	-20.11	22.97

Trait	QTL	Marker	Source	2001 G	2002 G	2003 G	2002 H	2003 H	LOD	A%	PV%
Plant height	*ph2.2*	GBMS216	Brenda	ns	***	ns	***	ns	4.14	-13.84	9.59
	ph3.1	Bmag603	HS584	ns	*	ns	**	**	2.78	10.91	6.55
	ph3.2	GBMS128a	HS584	ns	*	ns	**	***	3.31	16.11	8.09
	ph3.3	Bmag606	HS584	**	****	**	****	****	5.46	15.33	11.99
	ph4.1	Bmag490	HS584	ns	**	*	**	*	2.45	8.01	5.56
	ph5.1	Bmag223	HS584	ns	*	ns	*	*	1.40	5.18	3.5
	ph5.2	EBmac824	HS584	*	**	ns	ns	*	2.49	13.82	5.65
	ph7.1	Bmag516	Brenda	ns	**	*	**	*	2.73	-5.92	6.44
	ph7.2	GBMS128c	Brenda	ns	*	*	**	*	2.04	-3.46	4.84
	ph7.3	GBMS183	HS584	ns	*	ns	*	***	3.98	22.00	9.63
Ear length	*el2.1*	GBMS229	Brenda	*	****	****	****	**	7.90	-17.81	17.52
	el3.1	Bmag877	Brenda	ns	ns	ns	ns	****	4.78	-23.69	11.44
	el3.2	GBMS128a	HS584	ns	**	*	*	**	2.93	11.26	6.61
	el5.1	EBmac684	Brenda	*	***	ns	**	**	3.25	-14.96	7.32
	el7.1	GBMS240	Brenda	ns	**	*	***	*	4.31	-11.96	9.98
	el7.2	Bmag516	Brenda	ns	*	ns	*	****	4.42	-11.32	10.63
	el7.3	GBMS128c	Brenda	*	****	ns	*	ns	4.82	-5.92	10.65
Spikelet No. per ear	*sps1.1*	GBMS37	HS584	ns	*	ns	*	**	2.33	6.55	5.75
	sps2.1	GBMS229	Brenda	*	****	****	****	***	17.86	-20.84	36.52
	sps2.2	GBMS230	Brenda	**	****	*	****	**	6.93	-20.36	14.95
	sps2.3	GBMS216	Brenda	ns	***	****	***	ns	6.00	-14.62	14.16
	sps3.1	Bmag877	Brenda	ns	ns	ns	ns	***	4.37	-24.21	10.52
	sps5.1	EBmac684	Brenda	*	****	ns	****	***	7.33	-20.69	16.36
	sps7.1	Bmag516	Brenda	ns	**	**	ns	****	5.55	-13.43	13.17
	sps7.2	GBMS240	Brenda	ns	****	ns	****	***	6.64	-14.62	14.94
	sps7.3	GBMS35	Brenda	ns	****	ns	****	**	5.56	-17.86	12.19
	sps7.4	GBMS128c	Brenda	ns	****	*	**	ns	5.97	-6.17	13.04
	sps7.5	EBmag757	Brenda	*	*	**	*	*	2.88	-5.19	7.07
Grain No. per ear	*gps1.1*	GBMS37	HS584	*	*	ns	*	**	2.80	5.97	6.87
	gps2.1	GBMS229	Brenda	*	****	****	****	**	21.49	-23.62	42.12
	gps2.2	GBMS160	Brenda	ns	***	*	****	***	6.73	-18.37	15.12
	gps2.3	GBMS216	Brenda	ns	*	****	**	*	7.21	-17.06	16.75
	gps5.1	EBmac684	Brenda	*	***	ns	****	***	7.09	-20.00	15.88
	gps6.1	EBmac674	Brenda	*	*	ns	ns	***	2.69	-13.72	6.61
	gps7.1	EBmac603	Brenda	ns	***	ns	***	**	3.87	-17.84	8.65
	gps7.2	Bmag516	Brenda	ns	*	*	*	*	1.75	-2.34	4.36
	gps7.3	GBMS128c	Brenda	ns	**	*	*	**	2.77	-4.26	6.27
	gps7.4	EBmag757	Brenda	ns	**	**	*	*	2.65	-4.84	6.51
	gps7.5	HvPLASCIB	HS584	*	*	*	*	****	4.60	-12.24	11.04
Spike No. per plant	*spk3.1*	Bmag603	Brenda	*	*	nd	nd	nd	1.12	-22.94	2.59
	spk6.1	GBMS180	Brenda	ns	**	nd	nd	nd	2.17	-26.73	4.97
	spk7.1	GBMS183	Brenda	**	**	nd	nd	nd	2.27	-33.42	5.19
1000-grain weight	*tgw1.1*	GBMS14	Brenda	**	*	***	*	nd	3.30	-9.70	8.37
	tgw1.2	Bmag718	Brenda	*	ns	***	ns	nd	4.23	-5.37	10.66
	tgw2.1	GBMS137	HS584	*	*	***	ns	nd	4.37	-10.85	10.87
	tgw3.1	Bmag606	HS584	*	*	ns	***	nd	4.22	15.42	9.87
	tgw3.2	EBmac541	HS584	*	*	ns	*	nd	1.56	6.93	3.77
	tgw4.1	GBMS81	Brenda	****	****	ns	ns	nd	4.78	6.40	10.66
	tgw5.1	GBMS119	Brenda	**	**	**	**	nd	2.73	-10.55	6.94
	tgw6.1	GBMS222	Brenda	***	***	****	**	nd	4.70	-4.51	11.64
	tgw7.1	GBMS240	Brenda	**	**	****	ns	nd	8.44	-13.68	19.92
	tgw7.2	Bmag341	Brenda	****	***	***	*	nd	4.67	-12.29	10.55
	tgw7.3	EBmag757	Brenda	***	***	***	***	nd	4.25	-10.28	9.95
	tgw7.4	GBMS128b	HS584	***	***	ns	**	nd	3.25	5.09	7.39

Trait	QTL	Marker	Source	2001 G	2002 G	2003 G	2002 H	2003 H	LOD	A%	PV%
Powdery mildew R.	*ml4.1*	EBmac701	Brenda	nd	nd	nd	****	ns	8.46	-14.56	18.81
	ml5.1	Bmag387	Brenda	nd	nd	nd	****	****	7.59	-11.96	17.39
Leaf rust R.	*rph3.1*	Bmag606	Brenda	nd	nd	nd	*	*	1.92	-1.67	4.72

Significance levels: * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$
nd: no data; ns: not significant.
Regions of the genome were identified as putatively containing a QTL with the method described in the materials and method section.
G = Gatersleben, H = Hadmersleben.
LOD score, A% and PV% from the case with the underlined *P*-value
The phenotypic change percentage of (A%) of each significant at a given maker locus, was calculated as (100*(BB-AA)/AA + 200*(AB-AA)/AA)/2, where AA is the phenotypic mean for individuals homozygous for variety alleles at specified markers and BB is the phenotypic mean for individuals homozygous for *Hordeum spontaneum*.
The percent phenotypic variance (%PV) associated with each significant QTL was calculated from the regressions of each maker / phenotype combination based on data from the case with the underlined *P*-value.

5.3.3 Discussion

5.3.3.1 Clustering of QTLs detected in this study

Similar to the accumulations of QTLs found on chromosomes 1H, 2H, 5H and 7H in the 'Brenda' × HS213 population, multiple QTLs were also observed in the respective genomic regions in the 'Brenda' × HS584 population. While 43 putative QTLs were detected in the 'Brenda' × HS213 population, 81 putative QTLs were identified in the population 'Brenda' × HS584. In the population 'Brenda' × HS584, 26 putative QTLs for all investigated traits covered the entire length of chromosome 7H, while only six QTLs including one cluster of four QTLs were identified on the same chromosome in 'Brenda' × HS213. While a large marker gap existed on the long arm of chromosome 3H in the 'Brenda' × HS213 map, eight QTLs were mapped in the same region based on eight polymorphic microsatellites in 'Brenda' and HS584. A cluster consisting of four QTLs was verified in the two populations on chromosome 3HL near to the region of the *denso* gene (Thomas *et al.* 1991 and 1995; Barua *et al.* 1993;). In both cases, the highest number of detected QTLs were on the short arm of chromosome 2H in the interval of *GBMS2* and *GBMS229*. The allele from the recurrent parent 'Brenda' in this region affected strongly the performance of most agronomic traits.

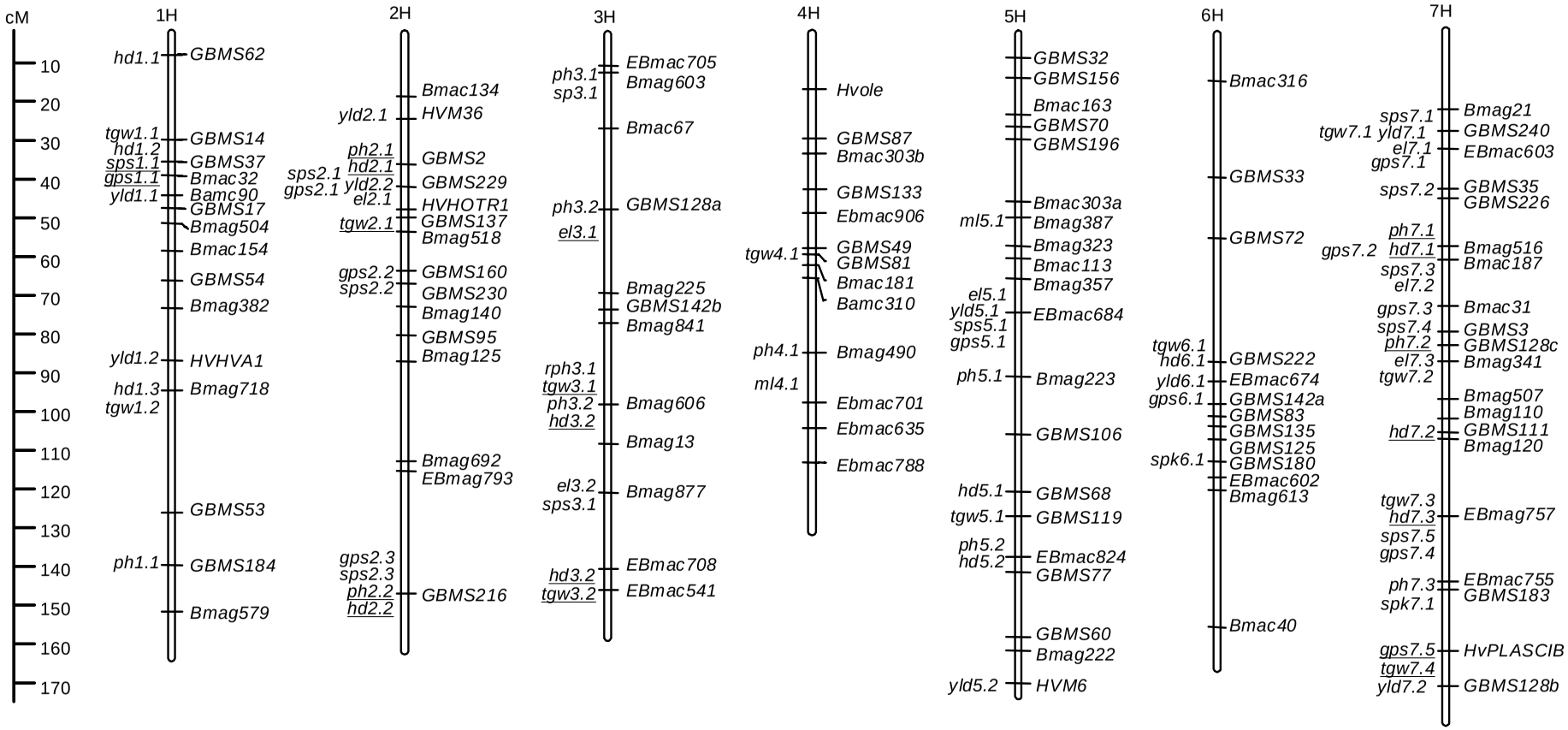


Fig. 5.6 Map locations of putative QTLs in the Brenda/HS584 population. The order of markers and the distances in cM (Kosambi mapping units) has been estimated and based on the barley molecular map (Li et al., 2003; Ramsay et al., 2000; Liu et al., 1996). The centiMorgan scale is given on the left. Locus name is indicated on the right side of the chromosomes. QTLs are designated by the *letter/number combination* to the left of the chromosomes. *Underlined* QTL: the allele from HS584 is favourable for the trait. Abbreviations for traits are listed in Table 5.7.

5.3.3.2 Comparisons of the AB-QTL analyses between 'Brenda' × HS213 and 'Brenda' × HS584 with other QTL analyses in barley

Two advanced backcross populations were assessed at the same two locations during a period from 2000 to 2003. Different wild barley accessions, HS213 and HS584 were used as the donor parents, respectively. Different performances of the donor segments form the two wild barleys in the same genetic background were identified with both population sharing an elite spring barley variety 'Brenda' as the recurrent parents. The size of the two populations was similar with more than 200 individuals. Table 5.11 presents the numbers of the polymorphic markers surveyed and the putative QTLs identified in both populations, respectively. Whereas the population 'Brenda' × HS213 population was genotyped with only 60 polymorphic microsatellites, the number of markers surveyed in the 'Brenda' × HS584 population increased to 107. The number of putative QTLs detected in the populations was 77 in the 'Brenda' × HS584 compared to 43 in the 'Brenda' × HS213. Most of QTLs detected in the population 'Brenda' × HS584 were located on chromosome 7H. This may be associated with the fact that twice the number of the polymorphic markers was genotyped on the chromosome 7H in the 'Brenda' × HS584 compared to the 'Brenda' × HS213 population. Ten and 18 QTLs of wild species loci carrying positive effects were located in the two populations, respectively (Table 5.11). Nine QTLs from 'Brenda' were confirmed and reproducible in both populations. This finding revealed that the effects of these alleles were reliable and their performance was not affected by environmental factors. With the exception of one QTL on chromosome 2HS for heading date, most QTLs alleles of the two different donor parents were found at identical or similar locations on the chromosomes.

Table 5.11 Numbers of markers used and putative QTLs detected in two populations

Population		Chromosome							
		1H	2H	3H	4H	5H	6H	7H	Total
'Brenda' × HS213 population	No. of markers	6	11	4	14	10	5	10	60
	Putative QTLs	6	18	1	1	9	2	6	43
	QTLs from HS213	2	4	0	1	1	1	1	10
'Brenda' × HS584 population	No. of markers	15	15	12	12	30	13	20	107
	Putative QTLs	10	14	10	2	10	5	26	77
	QTLs from HS584	2	3	5	0	0	0	8	18
Common QTLs	QTLs from Brenda	1	5	0	0	4	1	2	9

5.3.3.2.1 QTLs for yield

Five and nine yield-increasing QTLs were mapped in the populations 'Brenda' × HS213 and 'Brenda' × HS584, respectively. For all of them, the alleles from the common parent 'Brenda'

had a positive effect. In the population 'Brenda' × HS584, the QTLs *yld1.1*, *yld2.2* and *yld6.1* were verified in similar regions on the same chromosomes in the 'Brenda' × HS213 population. The failure of confirmation of the remaining yield QTLs in the 'Brenda' × HS584 population was possibly due to a lack of common makers in the two populations. Similar results were obtained in a comparative AB-QTL analysis by Pillen *et al.* (2004) with identical donors in different genetic backgrounds. The QTL, *yld2.2* located on chromosome 2HS was in both populations most probably caused by the pleiotropic gene *Ppd-H1* (Laurie *et al.*1994).

5.3.3.2.2 QTLs for heading date

Whereas all eight alleles from HS213 reduced the days to heading, of thirteen putative QTLs detected in the 'Brenda' x HS584 population, six alleles from wild species increased the number of days to heading. A QTL, *hd1.3* was mapped on the long arm of chromosome 1H, to a similar position as the flowering time gene *Ppd-H2* described by Laurie *et al.* (1995). The effects of the HS584 allele (*hd2.1*) on chromosome 2HS had a major effect on flowering time and were consistent with wild species alleles found on the same region in the 'Brenda' × HS213 population. It can be presumed that both wild species carried the same QTL decreasing the number of days to heading. As discussed in chapter 5.2.3.2.2, this common QTL might be a candidate of *Ppd-H1* photoperiodism gene. Of five QTLs of smaller effect for earliness detected in the study by Laurie *et al.* (1995), two (*eps3L* and *eps6L*) were mapped on chromosomes 3HL and 6HL. Two QTLs were found in the Brenda × HS584 lines at similar positions. In this study, the effects of the donor segments at these two loci were contrary. For the QTL on chromosome 3HL, *hd3.2* the HS584 allele had a positive effect on earliness decreasing the days to flowering. The other QTL on chromosome 6HL, *hd6.1* of donor segment increased the number of days to heading. Based on 58 near isogenic lines in the 'Brenda' × HS584 population, a set of DH lines will be developed and will be available to examine the specific effects of these QTLs.

5.3.3.2.3 QTLs for plant height and lodging

Compared with only two QTLs detected in the 'Brenda' × HS213 population, twelve putative QTLs were mapped on the whole genome except chromosome 6H in the 'Brenda' × HS584 population. The HS213 alleles had opposite effects on plant height. The QTLs for plant height differed between the two populations. The QTL with the main effect, *ph2.1* of the HS584 allele colocated with positive effects of *hd2.1* at the same locus. A QTL, *ph3.2* mapped on chromosome 3HL. Here, *denso*, a dwarfing gene controlling plant height has been identified

at a comparable region by Barua *et al.* (1993), Laurie *et al.* (1994) and Bezant *et al.* (1996). The *denso* dwarfing gene is a major gene that is carried by many varieties currently used in barley breeding. A QTL, *ph7.3* was associated with *Ebmac755* on the long arm of chromosome 7H in the 'Brenda' × HS584 population. For the same trait, a QTL linked to the same microsatellite was reported by Pillen *et al.* (2003) in a BC_2F_2 population.

5.3.3.2.4 QTLs for ear length and spike number per plant

Concerning ear length, seven QTLs were assigned to barley chromosomes 2HS, 3HL, 5HL, 7HS and 7HL in the 'Brenda' × HS584 lines. Two QTLs, *el2.1* and *el5.1* could be found on short arms of chromosomes 2H and 5H near *GBMS229* and *Ebmac684* as *el2.1* and *el5.1*. They were related to the QTLs in the 'Brenda' × HS213 population. Thus, the consistence of effects from the recurrent parent alleles at these two loci were confirmed in both populations. For the remaining QTLs mapped in the 'Brenda' × HS584 population, no corresponding QTLs were found in the 'Brenda' × HS213 lines. While no QTLs for number of spike per plant were detected in the 'Brenda' × HS213 population, three QTLs for this trait were mapped on chromosomes 3H, 6H and 7H with low significance level in the 'Brenda' × HS584 lines.

5.3.3.2.5 QTLs for spikelet number per ear and grain number per ear

Eleven QTLs increasing the spikelet number per ear were detected on chromosomes 1H, 2H, 3H, 5H and 7H in the 'Brenda' × HS584 population. Most of them shared the same position with the QTLs for grain number per ear in the same cross. The average level of significance for each QTL for spikelet number per ear was higher than that of QTLs for grain number per ear. Compared to the effect of spikelet number on yield, the character of grain number per ear had a direct effect on total yield. Nevertheless, most QTLs for these two traits were observed to share the same position on each chromosome. This was also consistent with correlation results between the two characters (Table 5.7). In the 'Brenda' × HS213 population, seven and five putative QTLs were identified for these two traits, respectively. Generally, the QTLs from the Brenda alleles showed positive effects in both populations with some exceptions. For instance, for a QTL located on the chromosome 1HS, the Brenda allele was associated with significant but opposite QTL effect in the two populations. Similar phenomena were also described in a study by Pillen *et al.* (2004) to predict the performances of the donor alleles in different genetic background by using two related backcross populations. In most cases, the effects of QTLs from the recurrent parent had the same directions for each character in the

two populations, especially for the QTLs presented on the short arms of the chromosomes 2H, 5H and 7H.

5.3.3.2.6 QTLs for thousand grain weight

In the present study, twelve QTLs were found for thousand-weight in the 'Brenda' × HS584 population and one third were mapped to both arms of chromosome 7H. Four QTLs for increasing the 1000-grain weight originated from the wild species HS584 and were allocated to chromosomes 2H, 3H and 7H. A QTL with the 'Brenda' allele increasing 1000-grain weight detected in the population 'Brenda' × HS213 on chromosome 2HL was missing in the 'Brenda' × HS584 lines. QTLs detected on chromosomes 2H and 4H in the 'Brenda' × HS584 population were in similar positions to the 1000-grain weight QTLs reported by Pillen *et al.* (2003).

5.3.3.2.7 QTL for resistance to powdery mildew

Two QTLs for powdery mildew resistance were found on the long arm of chromosome 4H and the short arm of 5H in the population 'Brenda' × HS584, respectively; together they explained over 35% of the variance. The resistance allele of these two QTLs originated from the recurrent parent. By comparisons with common markers in four maps (Li *et al*. 2003; Ramsay *et al*. 2000; Schönfeld *et al* 1996; Barley BIN map, http://barleygenomics.wsu.edu/databases/databases.html), *ml5.1* was located in BIN7. The resistance gene *Mlj* was also mapped in same region (Schönfeld *et al*, 1996). Therefore, the effects of *ml5.1* on resistance to powdery mildew might be caused by the gene *Mlj*. It is reasonable to assume that the resistance found on chromosome 5HS corresponds to the one detected by Heun (1992). The other QTL on chromosome 4H, *ml4.1* was linked to *Bmag490*. Two resistance genes, *Mlg* and *mlo* were mapped in BIN 7 and 9 groups on chromosome 4H, respectively (Büschges *et al.* 1997; Kurth *et al.* 2001). The gene, *Mlg* was identified by tight linkage to RFLP marker *MWG058*. The markers *Bmag490* and *MWG058* were positioned in the same BIN7. Thus, the effects of *ml4.1* might be explained by the gene *Mlg*. So far, besides the gene *Mlg*, *mlo* and *Mlj* mapped to chromosome 4H and 5H resistance genes to powdery mildew of barley were identified on other chromosomes, such as *Mla* mapped to chromosome 1H (Schüller *et al*, 1992), *MlLa* to 2H (Hilbers *et al*, 1992), *Mlh* to 6H (Jorgensen *et al*, 1994), *Mlf* and *Mlt* to 7H (Schönfeld *et al*, 1994).

5.3.3.2.8 QTL for resistance to leaf brown rust

For resistance to leaf brown rust, a QTL was detected on chromosome 3H with low significance level. The 'Brenda' allele had a positive effect to disease resistance. So far, the number of putative resistance genes to leaf rust increased to 17 (Chelkowski *et al.* 2003). In *H. spontaneum* Feuerstein *et al*. (1990) were able to identify isozyme loci linked to a resistance genes, *Rph10* at BIN 13 group on chromosomes 3HL. The QTL found in the present study was linked to *Bmag606*, which belongs to BIN 12 group on the long arm of the 3H. Thus, the minor effects of *rph3.1* on resistance to leaf brown rust may result from an *Rph10* allele.

6 Conclusions and outlooks

The importance of barley (*Hordeum vulgare*) as a crop plant has prompted widespread genetic research onto this species. More than a thousand genes are known and several genetic maps and physical maps have been constructed. In this study, 127 new microsatellites originating from genomic DNA were mapped onto seven barley chromosomes. The polymorphism of these PCR-based markers was verified by a set of six barley varieties and three wild species accessions. The average PIC value was beyond 0.60 up to 0.94. These new primer pairs were integrated into two established barley linkage maps using the mapping populations 'Steptoe' × 'Morex' and 'Igri' × 'Franka', respectively. The chromosomal assignment of 48 mapped loci that were corroborated on a set of wheat-barley addition lines. Eighteen additional loci were not polymorphic between two parents for each mapping population were assigned to chromosomes by this method.

A total of 133 microsatellite loci as located on all seven linkage groups with four significant clusters in the centromeric regions of 2H, 3H, 6H and 7H. In total, 78 loci were integrated in the S/M map and 53 loci in the I/F map, while 16 shared in both maps. The comparison of microsatellites derived from genomic DNA libraries and from EST libraries identified higher polymorphism for those from genomic libraries. The improved coverage of the barley genetic map with microsatellite markers will facilitate the mapping of genes and QTLs which are of economic importance in barley, and support studies of genetic diversity, pedigree analysis and the display of graphical genotypes.

In this study, forty-two and eighty-one QTLs for agronomically important traits and malting quality were detected in two AB-populations, respectively. These two AB-populations shared the same recurrent parent 'Brenda', which was a German spring barley elite variety. Most QTL with positive effects originated from 'Brenda'. The donor parents were HS213 and HS584, respectively. Sixty and one-hundred seven polymorphic microsatellites were used to genotype the entire genome for each population.

A QTL originating from the wild species was found to decrease days to heading and mapped on the short arm of chromosome 2H in the BC_3-DH population 'Brenda' × HS213. A nearly isogenic line carrying this locus was obtained to produce a F_2 population by backcrossing to

'Brenda'. Then, the precise position of this favourable QTL was identified in the F_2 population as a single gene. The QTL was presumably the *Ppd*-H1 gene. This demonstrates that AB-QTL analysis can be applied to identify valuable QTLs in barley wild species and unadapted germplasm and selectively introduce and transfer them into elite varieties. This method with unbalanced populations minimizes the time from QTL discovery to variety release. Meanwhile, QTL-NILs offer materials to develop mapping populations in an uniform background for fine mapping these single QTLs as single Mendelian factors and will help to improve elite breeding lines.

A total of 34 nearly isogenic lines containing one single donor segment were found in the lines of the advanced backcross population 'Brenda' × HS213, which can be regarded as defined introgression lines. The reconfirmation of several QTLs detected on chromosome 1H and 5H for heading date is underway in several F_2 populations derived from the respective introgression lines carrying donor segments and 'Brenda'. The establishment of these F_2 populations in a homogenous genetic background will allow to map QTLs of interest precisely, with the prospect of a further map-based cloning approach.

In the second BC_3 population 'Brenda' × HS584, with more than one hundred polymorphic markers, fifty-five nearly isogenic lines were available from more than two hundred lines by genotyping. Based on these NILs, a population consisting of doubled haploid lines will be available in the future and can be used to reconfirm and investigate those effects of eighty one QTLs detected in this study. These QTLs were identified for eleven agronomic traits, which were evaluated at two locations over three years. Most positive effects for the trait yield were detected from the recurrent parent. In contrast, for the heading date, of thirteen QTLs found in total, seven alleles from the wild species were identified to reduce the days to heading. Nine QTLs originating from 'Brenda' were confirmed in both populations, including *hd2.2* detected in the 'Brenda' × HS213 population, which corresponded to the same locus found in the 'Brenda' × HS584. Once the BC_3-DH population will be obtained in the spring of 2005, analysis based on the homozygous donor segments will be helpful to identify and further map the QTLs identified in the current study.

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In 6th Gatersleben Research Conference, 7-11 March, 2002, Chateau Meisdorf/Harz, p.26

ERKLÄRUNG

Hiermit erkläre ich, daß dieser wissenschaftlichen Arbeit noch keine vergeblichen Promotionsversuche unternommen wurden.

Die eingereichte Dissertation habe ich selbständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt.

Des weiteren erkläre ich, daß keine Strafverfahren gegen mich anhängig sind.

Gatersleben, den 15. Juni 2004

Jingzhao Li