Aus dem Institut für Pflanzenzüchtung und Pflanzenschutz

Advanced Backcross QTL analysis and genetic study of an introgressed powdery-mildew resistance gene derived from *Avena macrostachya* in oat (*Avena sativa*)

Der Landwirtschaftlichen Fakultät
der
Martin-Luther-Universität
Halle-Wittenberg

als
Dissertation

zur Erlangung des akademischen Grades
doctor agriculturarum (Dr. agr.)

vorgelegt von

M.Sc. in Bio.
Jianzhong Yu

geb. am 21.01.1976
in Hubei, Volksrepublik China

Gutachter: Prof. Dr. W.E. Weber
Prof. Dr. P. Wehling
PD Dr. S. Maak

Verteidigung am: 24.10.2005

Halle/Saale 2005

urn:nbn:de:gbv:3-000009169
[http://nbn-resolving.de/urn/resolver.pl?urn=nbn%3Ade%3Agbv%3A3-000009169]

Table of Contents

Abbreviations

2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
AB	advanced backcross
AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
APR	adult plant resistance
bp	basepair
BSA	bulked segregant analysis
BYDV	barley yellow dwarf virus
CIM	composite interval mapping
cM	centiMorgan
CTAB	cetyltrimethylammonium bromide
DNA	deoxyribonucleic acid
DWD	deutscher wetterdienst
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
FIA	flow injection analysis
HPLC	high performance liquid chromatography
LOD	logarithm of odds
MAS	marker assisted selection
NIL	near isogenic line
NIRS	near infrared reflectance spectroscopy
OMR	oat mildew resistance
PAGE	olyacrylamide gel electrophoresis
PCR	polymerase chain reaction
QTL	quantitative trait locus
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RIL	recombinant inbred line
SIM	simple interval mapping
SNP	single nucleotide polymorphism
SPA	single point analysis
SSR	simple sequence repeat
STS	sequence-tagged-site
TE	Tris/EDTA
Tris	2-Amino-2 (hydroxymethyl)-1,3-propandiol
USDA	united states department of agriculture

1 Summary

1.1 Oat AB-QTL analysis

Oat (*Avena sativa*) is an important cereal crop which has high nutritional value due to its valuable protein composition, salubrious relation of unsaturated to saturated fatty acid, a high fibre content, and especially for its high concentration of mixed-linked (1-3, 1-4)-*β*-D-glucan, referred to as *β*-glucan. Advanced backcross QTL (Quantitative Trait Loci) analysis, developed by Tanksley and Nelson (1996), is a novel strategy which combines the mapping of interested QTL alleles and the introgression of these favorable alleles from exotic germplasms into one process. This method has been successfully applied in various crops such as wheat, barley, rice and tomato. However, no advanced backcross QTL research has been reported in oat.

In order to develop oat lines with favorable agronomic characteristics, particularly with high *β*-glucan content, AB-QTL analysis was performed in a BC_2F_2 population consisting of 98 lines derived from a cross of 'Iltis' x IAH611-447, in which the high yield but low *β*-glucan content cultivar 'Iltis' was used as recurrent parent while the unadapted high *β*-glucan line IAH611-447 was used as donor parent. A total of 144 oat SSR primer pairs and 256 AFLP selective primer pair combinations were used for genetic map construction and for QTL analysis. Phenotypic data of 11 traits from $BC_2F_{2:5}$ and $BC_2F_{2:6}$ lines at three locations in two years were evaluated for QTL identification.

110 loci were characterized on the 98 individuals of the BC_2F_2 population, resulting a map of 455cM with 12 linkage groups. 60 significant QTLs were detected for all 11 traits by simple interval mapping (SIM) method, ranging from 2 to 9 QTLs/trait. Most QTLs (67%) were detected in at least 2 environments. 4 QTLs were detected to be significantly associated with *β*-glucan content. All the 4 QTLs associated with *β*-glucan content have favorable alleles from the donor parent IAH611-447, explaining the phenotypic variation from 11.9% to 44.2%.

Present results indicate that some favorable QTL alleles contributing to high *β*-glucan content have been successfully introgressed from donor parent IAH611-447 into the elite oat cultivar

'Iltis'. The results provide helpful information for further oat breeding programmes.

1.2 Genetic study of an introgressed powdery-mildew resistance gene derived from *Avena macrostachya* in oat (*Avena sativa*)

Powdery mildew, caused by *Erysiphe graminis* D.C.f. sp. *avenae* Em. Marchal, is an important foliar disease of cultivated oat worldwide, especially in humid regions such as maritime northwest Europe and along the Atlantic seaboard. Several sources with resistance to powdery-mildew have been reported. However, only a few sources have been used in practical oat breeding and application of new stable sources of resistance would be helpful.

Avena macrostachya is a perennial and cross-fertilizing wild oat species, possessing several traits with high relevance for oat breeding, including the winter hardiness, the resistances to Barley Yellow Dwarf Virus (BYDV), and the powdery-mildew resistance. In this study, a genetic analysis of resistance to powdery mildew was conducted, based on two introgression lines of the cross ((*A. magna* x *A. macrostachya*) x *A. sativa* with powdery-mildew resistance derived from *A. macrostachya*. Both lines were crossed with susceptible cultivars 'Neklan' and 'Flämingsprofi' to generate F_1, F_2, $F_{2:3}$ and BC_1 families.

The powdery mildew tests revealed that the resistance is controlled by a dominant gene, tentatively designated *Eg5*. Molecular marker analysis was conducted using bulked-segregant analysis in two segregating F_3 populations. One codominant simple sequence repeat (SSR) marker and 20 AFLP markers were found to be tightly linked to the resistance gene. For high-throughput analysis in MAS breeding, 8 AFLP markers which gave clear polymorphic patterns were selected for PCR-based markers development. 4 AFLP-derived PCR-based markers were successfully developed. Linkage analysis revealed all these molecular markers were tightly linked with the resistance gene in both segregating populations.

Comparative mapping was conducted using 71 F_{10} recombinant inbred lines from the 'Kanota' x 'Ogle' reference mapping population (Wight et al., 2003). The linked SSR marker AM102 was mapped on the KO-22_44+18 linkage group, with a genetic distance of 1 cM to RFLP marker cdo419, and 3 cM to cdo484a.

Summary

In current work, the genetic analysis of the resistance to powdery mildew derived from *A. macrostachya* is performed for the first time. The new resistance source derived from *A. macrostachya*, together with the tightly linked PCR-based markers identified here, should be useful for both marker-assisted selection breeding purpose and further genetic studies of powdery-mildew resistances in oat.

2 Zusammenfassung

2.1 AB-QTL-Analyse bei Hafer

Der Saathafer (*Avena sativa*) ist eine bedeutende Kulturpflanze, die seit Jahrtausenden sowohl in der menschlichen Ernährung als auch in der Tierfütterung genutzt wird. Der ernährungsphysiologische Wert das Hafers wird durch eine wertvolle Proteinkomposition, das günstige Verhältnis ungesättigter zu gesättigten Fettsäuren und den hohen Ballastsstoffgehalt determiniert. Wie auch bei Gerste besteht letzterer zum Großteil aus (1-3, 1-4)-β-D-Glucan, dem Hauptbestandteil der Endospermzellwände. Nachweislich werden β-Glucan zahlreiche gesundheitliche Wirkungen zugeschrieben, wie die Senkung des Serumcholesterinwertes bei Hypercholesterin, eine regulierende Wirkung auf den Blutzuckerspiegel und somit eine Risikoverminderung für kardiovaskuläre Krankheiten.

Bei der sogenannten AB-QTL-Analyse ("Advanced Backcross QTL-Analysis"), vorgeschlagen durch Tanksley und Nelson (1996), werden interessante QTLs (Quantitative Trait Loci) aus Wildarten oder exotischen Kulturarten identifiziert, kartiert und in Hochleistungsmaterial überführt. Diese Methode wurde erfolgreich in den Gattungen *Triticum*, *Hordeum*, Oryza und *Lycopersicon* angewendet, fand jedoch noch keine Nutzung bei Hafer.

Um leistungsfähige Haferstämme mit besonders hohem β-Glucan zu entwickeln, wurde die β-glucanreiche Genbankherkunft IAH611-447 zweifach mit der ertragreichen Hafersorte 'Iltis' zurückgekreuzt. Zur Genotypisierung der 98 BC_2F_2-Individuen wurden 144 Hafer-Mikrosatelliten und 256 AFLP-Primerpaare eingesetzt. Von den $BC_2F_{2:5}$ und $BC_2F_{2:6}$ Linien wurden 11 agronomische sowie Qualitätsmerkmale an 3 Orten in den Jahren 2003 und 2004 erfasst und für die QTL-Detektion verrechnet.

Anhand der Spaltung zwischen den 98 BC_2F_2-Individuen wurden 110 Loci in 12 Kopplungsgruppen bei einer Kartengesamtlänge von 455cM kartiert. Insgesamt wurden 60 signifikante QTLs auf der Basis des Simple-Interval-Mapping-Methode (SIM) für alle 11 Merkmale detektiert, bei 2 bis 9 QTL je Merkmal. Die Mehrzahl der QTL wurde unter mindestens 2 Umwelten bestätigt. Vier QTLs für hohen β-Glucangehalt basieren auf positiv

wirkenden Allelen vom Donor IAH611-447 und erklären 11,9% bis 44,2% der phänotypischen Variation.

Die Ergebnisse belegen, dass vorteilhafte QTLs für hohen *β*-Glucangehalt in den genetischen Leistungshintergrund von 'Iltis' übertragen wurden. Die gewonnenen Erkenntnisse werden in der aktuellen Züchtung von ertragreichen Qualitätshafersorten genutzt.

2.2 Genetische Charakterisierung der introgressierten Mehltauresistenz aus *Avena macrostachya* im Saathafer (*Avena sativa*)

Hafermehltau, verursacht durch *Erysiphe graminis* D.C.f. sp. *avenae* Em. Marchal, ist eine bedeutsame Blattkrankheit des Saathafers mit besonderer Relevanz in den maritim beeinflussten Gebieten Nordwesteuropas wie an der Atlantikküste. In Großbritannien stellt Mehltau die gefährlichste Haferkrankheit dar und kann Ertragsdepressionen von bis zu 39% verursachen. Aus ökonomischer und auch ökologischer Sicht wird in der Resistenzzüchtung der geeignetste Weg gesehen, um diese Krankheit zu kontrollieren und Ertragsverlusten vorzubeugen.

Eine Reihe von Resistenzquellen aus Saathafer, Wildhafer wie *A. barbata, A. strigosa*, *A. occidentalis*, *A. pilosa* und *A. sterilis* wurden bisher beschrieben. Da nur wenige dieser Quellen praktische Bedeutung erlangt haben, sind neue stabile Resistenzen in der Sortenzüchtung gefragt.

Avena macrostachya ist eine ausdauernde, fremdbestäubende Wildhaferart mit züchterisch wertvollen Eigenschaften wie Mehltauresistenz, Resistenz gegen Gerstengelbverzwergungs-virus und Winterhärte. In der vorliegenden Arbeit wurde die Mehltauresistenz aus *A. macrostachya* genetisch analysiert, wofür dem Autor zwei Introgressionslinien der Kreuzung ((*A. magna* x *A. macrostachya*) x *A. sativa* als Ausgangsmaterial für die genetische Analyse zur Verfügung gestellt wurden. Die Mehltauresistenz wurde an den F_1, F_2, $F_{2:3}$ und BC_1 aus Kreuzungen der Introgressionslinien mit den mehltauanfälligen Sorten 'Neklan' und 'Flämingsprofi' mittels Blattsegmenttest auf Benzimidazolagar geprüft. Anhand der Spaltungsergebnisse kann von einem monogen dominanten Erbgang der Mehltauresistenz aus

A. macrostachya ausgegangen werden. Das putative Gen wurde in Fortsetzung der internationalen Nomenklatur *Eg5* benannt.

Der nächste Schwerpunkt bestand in der Suche und Kartierung PCR-gestützter molekularer Marker mit enger Kopplung zur Mehltauresistenz. Hierfür wurde zunächst die sogenannte “bulked-segregant analysis” auf zwei F3-Populationen angewendet. Ein kodominanter Mikrosatellitenmarker sowie 20 AFLP mit enger Kopplung zur Resistenz wurden gefunden. Um Marker für spätere Hochdurchsatzanalysen im Rahmen der markergestützten Selektion zu entwickeln, wurden 8 AFLP mit besonders deutlichen Polymorphismen zur Ableitung von STS (Sequence-Tagged-Site)-Markern ausgewählt. Es konnten ein kodominanter und drei dominante STS gewonnen werden. Auch für diese Marker konnte eine enge Kopplung zur Mehltauresistenz in beiden spaltenden Populationen nachgewiesen werden.

Für eine vergleichende Kartierung der gefundenen Marker wurden 71 rekombinante F_{10}-Inzuchtlinien der Kartierungspopulation 'Kanota' x 'Ogle' (Wight et al., 2003) genutzt. Der Mikrosatellitenmarker AM102 wurde in der Kopplungsgruppe KO-22_44+18 mit einer genetischen Distanz von 1 cM zum RFLP cdo419 und 3 cM zu cdo484a kartiert.

Mit der vorliegenden Arbeit wird erstmalig eine genetische Analyse der Mehltauresistenz aus *A. macrostachya* vorgestellt, basierend auf Kreuzungen mit mehlauresistenten hexaploiden Introgressionslinien. Desweiteren wurde mit den eng zur Resistenz gekoppelten PCR-gestützten Markern erstmalig bei Hafer der Weg für eine markergestützte Selektion auf Mehltauresistenz bereitet. Die gewonnenen Erkenntnisse können sowohl für die markergestützte Selektion als auch für weiterführende genetische Studien der Mehltauresistenz von Hafer genutzt werden.

3 Literature review

3.1 Oat: an important cereal crop

Like other important crops, oat (*Avena sativa*) has been used as human diet and animal feed for several thousands years. Improving yield while maintaining good quality is always a long-term object for oat breeders.

The high nutritional value of oat is mainly attributed to its composition of protein, lipid and fibre (Ranhotra and Gelroth, 1995). After growing under the same low level of N-fertilizer, the concentration of protein in oat is higher than in other cereals. Oat protein is composed with higher amounts of several essential amino acids such as lysine, leucine, isoleucine and valine. Oat is also high in lipid composition which is uniformly distributed throughout the grain and has a favorable ratio of unsaturated to saturated fatty acids.

Oat flour and oat bran has considerable values as a source of protein, energy and phosphorus for nonruminant animals. It is richer in lysine, tryptophane and metabolizable energy than that of corn grain. However, oat flour is lower in both methionine and threonine compared with corn. Therefore, these two amino acids need to be carefully checked when formulating diets for swine and poultry (Hahn et al., 1990).

3.1.1 Oat *β*-glucan: physiological values

In oat, the mixed-linked (1-3, 1-4)-*β*-D-glucan, referred to as *β*-glucan, is a major component of the endosperm cell walls (Burke et al., 1974). As a high-molecular weight water-soluble fibre, *β*-glucan contributes to the main health benefits of oat for that it lowers the serum cholesterol levels of blood and balances the glucose and insulin contents of serum after meals, reducing the risks of cardiovascular diseases (Van Horn et al., 1988; Shinnick et al., 1991). The physiological effects are probably related to the gel forming properties of *β*-glucan, which increase viscosity of intestinal chyme. And increased viscosity disturbs micelle formation, which may inhibit cholesterol absorption, slow cholesterol transfer across the unstirred layer, and increase bile acid excretion by inhibiting bile acid reabsorption (Colleoni-Sirghie et al., 2003). Oat *β*-glucan also possesses immunomodulatory activities

capable of stimulating immune functions both in vitro and in vivo (Estrada et al., 1997). Therefore, developing oat lines with high *β*-glucan content for potential uses in both human food and medical field is highly desired by plant breeders.

3.1.2 Oat *β*-glucan: contents and methods for analysis

There are significant variations for groat *β*-glucan content within cultivated oat (*Avena sativa*) and wild oat species. For *A. sativa*, groat *β*-glucan content ranges from 1.8-7.5% (Peterson, 1991; Welch et al., 1991; Lim et al., 1992; Welch, 1995; Cervantes-Martinez et al., 2001). Groat *β*-glucan content of most wild oat species ranges from 1.2-6.1% (Welch et al., 1991; Miller et al., 1993a). Welch et al. (2000) found that one *A. atlantica* accession had the highest *β*-glucan concentration of 11.3% among the wild and cultivated oats. This accession may be useful source for increasing the *β*-glucan content of cultivated oats.

Several methods have been used for determination of *β*-glucan content. Near Infrared Reflectance Spectroscopy (NIRS), a well-known technique used in the food and feed industry, is also used for oat *β*-glucan analysis. Except high establishing expenses, NIRS method is cheap, fast (less than 1 min per sample) and moreover, several components can be determined at the same time. Unfortunately, NIRS often gives a lower accuracy. Automated flow injection analysis (FIA) is another method for rapidly quantifying *β*-glucan content. The method is based on the measurement of intensity of fluorescent absorption of a dye-*β*-glucan complex. Lim et al. (1992) estimated the variability for *β*-glucan content measured by FIA and found that the FIA procedure gave consistent sample readings. The most accurate method for *β*-glucan analysis is an enzymatic method, based on a bacterial enzyme [(1-3, 1-4)-*β*-D-glucan 4 glucanohydrolase] produced by *Bacillus subtilis*. However, the cost of this method is much higher compared to that of other methods, limiting its wide application.

3.1.3 Oat *β*-glucan: inheritance and correlated responses

Oat *β*-glucan content is a polygenic trait controlled by multiple genes with additive effects (Holthaus et al., 1996; Kibite and Edney, 1998). Several studies have been carried out to estimate heritability for *β*-glucan content. In a study of Humphreys and Mather (1996), heritability estimates for *β*-glucan content were between 0.27 and 0.45. In the studies of

Holthaus et al. (1996), Kibite and Edney (1998), the heritability estimates for *β*-glucan content ranged from 0.45 to 0.58. The highest heritability estimates for *β*-glucan content ranged from 0.80 to 0.85 on a line mean basis, reported by Cervantes-Martinez et al. (2001).

Oat *β*-glucan content is also affected by environmental factors. However, the relative importance of genotype x environment interaction is not very clear. All these high heritabilities reflect low genotype x environmental interactions. Holthaus et al. (1996) reported that the interaction of genotype x environment is minor among different environments, while Humphreys and Mather (1996) reported that the groat *β*-glucan concentration is strongly influenced by the environment and it is difficult for plant breeders to effectively select oat progeny with high groat *β*-glucan. Peterson (1991) also reported significant variation for *β*-glucan concentration among different locations. Although significant effects of genotype x environment interaction were found, for most cases, the rankings of oat genotypes on the basis of *β*-glucan content were similar over environments (Peterson, 1991; Brunner and Freed, 1994; Saastamoinen et al. 1992). Thus, it has been suggested that the selection of high *β*-glucan in a single environment should be representative of relative performance in other environments (Peterson, 1991).

In general, correlation-based responses may cause changes in agronomically important traits when direct selection for a single trait is practiced. Saastamoinen et al. (1992) reported *β*-glucan content had a significant positive correlation with grain yield, growing time, test weight and thousand-grain weight, but negative correlation with protein content and hull content. In contrast, Welch et al. (1991) suggested that there was a positive relationship between *β*-glucan content and protein content within a cultivar, but there was no significant overall correlation when a range of genotypes were included. There is no consistent correlation for oat *β*-glucan content with other agronomic traits (Cervantes-Martinez et al. 2002).

3.2 Genetic markers

3.2.1 Conventional genetic markers

There has been a long history for the development of genetic markers. The earliest genetic markers studied by biology scientists are phenotypic markers. In 1913, based on analysis for several phenotypic traits such as eyes colour, wings shape, bodies size and colours of the fruit fly, Alfred Sturtevant, an undergraduate student of Morgan, constructed the first linkage map in *Drosophila*. Since then, a lot of different phenotypic markers have been identified in several important crops like rice, soybean and tomato. However, the number of suitable phenotypic markers linked to interested genes is limited, which makes it difficult to construct a dense genetic map only with phenotypic markers. Also, some of the phenotypic markers are easily influenced by environments. All these drawbacks limit the widely application of phenotypic markers in plant breeding.

Cytological marker is another kind of genetic markers. Based on the polymorphisms of the structure and numbers of chromosomes, it is possible to localize genes on specific chromosomes. In wheat, monosomic and telosomic lines have been widely used to identify chromosomes carrying particular genes and to map these genes to the centromere. In rice, Khush et al. (1984) developed twelve primary trisomics lines and identified marker genes for each of the 12 chromosomes. Compared to phenotypic markers, cytological markers are less influenced by environments. The disadvantage of cytological markers is that the development of materials is both time and labour consuming. Additionally, some species are susceptible to the changes of chromosomes structures or numbers of chromosomes, limiting the application of cytological markers.

Proteins have been widely used as another kind of important genetic markers. There are two kinds of important protein markers: enzymatic protein marker and non-enzymatic protein marker. The most widely used non-enzymatic protein markers are seed storage proteins. These proteins can be easily separated and identified by different kinds of electrophoresis such as polyacrylamide gel electrophoresis (PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), or chromatographic techniques like high performance liquid chromatography (HPLC). In wheat, gliadins and glutenins consist 90% of seed storage proteins and have been used as important biochemistry markers for quality control and genetic analysis (Payne, 1987). After the first study of isoenzyme reported by Markert and Moller (1959), enzymatic protein markers, based on the staining of proteins with identical function

but different electrophoretic mobilities, have been widely applied as the most important genetic markers for genetic analysis prior to the development of DNA based markers. As the expression products of genes, proteins have more advantages used as genetic markers compared to phenotypic markers and cytological markers for that they are less influenced by the environment, and the development of protein markers is much easier. However, special staining methods are always required for most isoenzyme markers, and the number of protein markers is still not enough for constructing a high density map on genome wide which would be beneficial for plant breeding.

3.2.2 DNA-based molecular markers

All the problems related to traditional genetic markers mentioned above seem to be resolved since the development of DNA-based markers. There are several significant advantages for DNA-based markers. 1. DNA markers are phenotypically neutral, seldom influenced by environments, and fingerprinting based on DNA can be done at any stages of plant's growth; 2. Theoretically, there are numerous DNA markers, and these markers are distributed through the whole genome, which make it possible to get high density map on genome wide; 3. Some types of DNA markers have a high level of allelic polymorphism; 4. Only small amounts of plant tissue are needed for DNA operation, and always with highly reproducible fingerprint patterns; 5. DNA markers are more easily automated in handling, increasing both time and cost efficacies.

Several major types of DNA-based markers are described as following:

Restriction fragment length polymorphisms (RFLPs). RFLPs are hybridization-based DNA markers and were initially developed for human-genome mapping (Botstein et al., 1980). In plants, RFLPs allowed the construction of whole-genome maps for the first time (Bernatzky and Tanksley, 1986; Helentjaris et al., 1986). The polymorphisms of RFLPs depend on the point mutation, deletion, insertion, or transposition of DNA sequences. The DNA is digested by various restriction enzymes, separated through agarose gel electrophoresis and bounded to nylon membranes, following with hybridization of special DNA probes. The polymorphisms are detected by size differences of restriction fragments to

which the probe hybridize, and depends on the DNA probes/restriction enzymes combinations. RFLPs are highly efficient techniques for construction of high-density genetic maps, and are very useful for the analysis of comparative genomics. The major disadvantage for RFLPs is their time and labour handling. And for some plants, RFLPs always give low polymorphisms.

Random Amplified Polymorphic DNAs (RAPDs). RAPDs are PCR-based markers first described by Williams et al. (1990). The technique involves the utilization of a set of PCR primers containing 8-10 nucleotides whose sequence is essentially random. The polymorphisms depend on the sequence differences in primer sites or deletions/insertions of PCR products. The RAPD technique is easy and economical to use. No sequence information is needed and the polymorphisms can be detected through conventional agarose-gel electrophoresis. Despite the wide application of this technique for many crops, the poor reproducibility is a major disadvantage of RAPD. Up to 60% error was reported by Pérez et al. (1998). The problem can be partly resolved by converting RAPD fragments to conventional PCR markers such as sequence characterized amplified regions (SCARs), described by Paran and Michelmore (1993).

Microsatellites or Simple Seqence Repeats (SSRs). SSRs are tandem repeats of short nucleotide-sequence motifs and are flanked by conserved sequences. SSRs are abundant and relatively evenly distributed through eukaryotic genomes (Tautz and Renz, 1984). Condit and Hubbel (1991) reported the first study of SSRs in plants. One year later, Akkaya et al. (1992) reported the first length polymorphisms of SSRs in soybean. Since then, as a new PCR based markers, SSRs have been widely used for nearly all important crop plants. SSRs are highly polymorphic, easy to use, locus-specific and codominant markers which are particularly useful for mapping and genetic analysis. Despite the costs for SSR-marker development, a large number of SSR markers are already available for research for several important agricultural crops.

Amplified Fragment Length Polymorphisms (AFLPs). Developed by Vos et al. (1995), AFLP is a universal, multi-locus DNA marker technique. The technique is based on the selective PCR amplification of restriction fragments from digested genomic DNA. Briefly, genomic DNA is digested with specific restriction enzymes, and ligated to double-stranded

adapters with restriction site sequences as target sites for PCR reactions. AFLP is a highly efficient technique for its capacity to reveal many polymorphic bands in a single reaction. It is also highly reliable and reproducible. AFLP makers have been used as an attractive tool for the construction of whole-genome and high resolution maps in different plants.

Single Nucleotide Polymorphisms (SNPs). SNPs are single nucleotide positions where there are frequently variations of nucleotides in the population at a specific location of the genome. In the definition of a SNP, the stipulation is that DNA molecules must differ at the nucleotide side “frequently”. This provision excludes rare genetic variation of the sort found in less than 1 percent of the DNA molecules in a population. This is because that the genetic variants which are too rare are not generally as useful in genetic analysis as the more common variants. SNPs are the most common type of genetic variability found in the human genome with an estimated frequency of one for every 1.2-1.5 Kb. The binary character and stability from generation to generation make SNPs suitable for automated, high-throughput genotyping. Though there is not much information about the frequency of SNPs in plant genomes and very few reports of utilization of SNPs in plants, it is certain that SNPs will be the most attractive tool and the next generation of genetic markers for many plants in near future.

3.3 Advanced backcross QTL analysis

Most important agronomic and economic performances of crops, like yield, quality and certain resistances, are controlled by complex quantitative traits. Quantitative traits are controlled by several genes, and also influenced by the environment. For a long period, the study of quantitative traits was carried out with quantitative genetical approaches, without knowledge on the actual number and location of the genes that underlie the variances. Till recently, the development of molecular markers makes it possible to identify and genetically localize at least some of the contributing genetic factors as quantitative trait loci (QTL). Based on the molecular markers linked to QTLs, it is possible to trace these genetic factors during the breeding procedure and thus to utilize these QTLs for crop improvement.

Typically, QTL analysis involves two steps. The first step is to discover QTL, also called as

QTL mapping. The second step is to utilize QTL information to develop new varieties with high agronomic performance. In 1988, Paterson et al. reported the first QTL analysis in tomato by using a complete linkage map of restriction fragment length polymorphisms (RFLP). Since then, there has been increasing number of QTL studies in nearly all agronomic important crop species. However, despite these successes, the examples of utilizing molecular marker techniques to create new varieties with enhanced quantitative traits are limited. This limitation may be due to two points. 1. In the conventional approaches, the QTL mapping and variety development are separate processes. The most often used populations for conventional QTL analysis are F_2 or F_3 families, BC_1, and recombinant inbred lines. These are all suitable, efficient populations for QTL mapping. However, once valuable QTLs are identified in these populations, it is always necessary to take sequential backcrosses or intercrosses in order to get stable commercial varieties. 2. Alleles contributing to interesting quantitative traits identified in most QTL studies are limited and often already present in elite germplasms. In conventional breeding program, the QTL analysis is carried out in populations developed from a primary adapted pool of elite germplasm sources. Working within elite gene pool always gives successful selections. However, elite germplasm has reduced levels of genetic variation, making it difficult to find necessary polymorphism with the molecular markers which are required for QTL analysis, thus reducing the capacity of identifying more QTLs. Additionally, focusing only on elite germplasm always results in identifying the same alleles on which breeders have been worked for many years through classical breeding methods.

To overcome the drawbacks in conventional QTL analysis, Tanksley and Nelson (1996) reported a novel strategy: advanced backcross QTL analysis. They combined the mapping of interested QTL alleles and the introgression of these favourable alleles from exotic germplasms into one process by delaying QTL analysis until an advanced generation like BC_2 or BC_3. There are several advantages utilizing this method for QTL analysis as outlined by Tanksley and Nelson (1996): 1. Phenotypic selections can be conducted in early generations, reducing the frequency of undesirable alleles from the donor, and major negative QTLs can be reduced or eliminated. 2. The advanced generation is skewed towards alleles from the recurrent parent, reducing the possibility for detection of QTLs with epistatic interactions, but making it easier to detect additive QTLs which will still function in the nearly isogenic background of the recurrent parent. 3. The mean performance of the advanced generation is

skewed towards the elite parent, making subtle pleiotropic effects easier to be detected. 4. Only few additional backcross generations will be needed to create nearly isogenic lines with selected QTLs. Such QTL-NILs will be potential candidates for new varieties with enhanced attributes.

To date, advanced backcross QTL analysis has been successfully applied in several crops like wheat (Huang et al., 2003), barley (Pillen et al., 2003), rice (Xiao et al., 1998; Moncada et al., 2001) and tomato (Tanksley et al., 1996; Bernacchi et al., 1998). As to oat, no study regarding advanced backcross QTL analysis has been reported yet.

3.4 Oat genome and mapping

The genus *Avena* L. belongs to the tribe Aveneae of the Gramineae family. The species of the genus *Avena* form a polyploid series with different number of chromosome sets, but have a basic chromosome number of n = 7 (Leggett and Thomas, 1995). Four *Avena* genomes (A, B, C, D) have been identified and three ploidy levels are known as diploids where the chromosome number is 2n = 2x = 14 (AA, CC), tetraploids where 2n = 4x = 28 (AABB, AACC), and hexaploids where 2n = 6x = 42 (AACCDD). The cultivated oat *Avena sativa* (2n = 6x = 42) is a natural allohexaploid containing three genomes (A, C, D). It is suggested that the evolution of oat nuclear genomes involved cycles of interspecific hybridization and polyploidization. Although the precise diploid progenitors in the evolution of hexaploid oat are still not clear, it is highly probable that the A and C genome diploid species contributed these two genomes of hexploid oat (Leggett and Thomas, 1995). There is still no information available on the origin of the D genome, but several studies suggest that the A and D genomes are very closely related (Leggett and Markhand, 1995; Linares et al., 1996; Linares et al., 1998). Considering the absence of D genome diploid species, it appears possible that A genome diploid species could be the donor of both A and D genomes of hexaploid oat.

Compared to other crops like wheat, rice and barley, the mapping of the oat genome has lagged behind. This is partly due to the large genome size of oat and its polyploid nature. Particularly, unlike wheat, oat lacks a complete set of either nullisomic or nullitetrasomic lines,

which makes it difficult to localize molecular marker loci to specific chromosomes.

The first molecular linkage map of hexaploid oat was developed by O'Donoughue et al. (1995), using a recombinant inbred-line (RI) population from a cross between *Avena byzantina* C. Koch cv Kanota and *Avena sativa* L. cv Ogle. The map consisted of 561markers covering a distance of 1482 cM, with 38 linkage groups. Since then, several maps of hexaploid oat have been reported from the crosses Clintland64 x IL86-5698 (Jin et al., 2000), 'Ogle' x TAM O-301 (Portyanko et al., 2001), 'Kanota' x'Marion' (Groh et al., 2001a), and 'Ogle' x MAM17-5 (Zhu and Kaeppler, 2003a). As a fulcrum, the 'Kanota' x 'Ogle' map developed by O'Donoughue et al. (1995) has been used for various oat genomic studies.

Recently, based on the results from Jin et al. (2000) and Groh et al. (2001a), Wight et al. (2003) improved the previous KO maps with additional markers and RI lines. The map contained 1166 markers spanning 1890 cM of the oat genome. The 1166 markers produced 29 linkage groups, with 43 unlinked loci. This map is currently the most complete hexaploid oat molecular linkage map. The KO maps together with maps developed from other crosses (Table 3.1) can be used as useful references for oat genomic research.

3.5 QTL analysis in oat

Several studies of QTL analysis for important characters have been reported in oat. Using a population of 84 recombinant inbred lines derived from a cross between 'Kanota' x 'Ogle', Siripoonwiwat et al. (1996) detected QTLs for grain yield, test weight, groat percentage, straw yield, days to heading, and plant height. QTLs for grain yield, heading date and plant height were also detected by De Koeyer et al. (2001) in a recurrent selection population. QTLs for other important agronomic traits including hull percentage (Ronald et al., 1997), chemical composition and kernel characteristics (Kianian et al., 1999, 2000; Groh et al, 2001b), photoperiod and vernalization responses (Holland et al., 2002), resistance to crown rust (Zhu and Kaeppler, 2003b) and barley yellow dwarf tolerance (Jin et al. 1998; Barbosa-Neto et al., 2000; Zhu et al., 2003), were also reported in oat.

Table 3.1 Summary of hexaploid oat molecular linkage map and marker

	O'Donoughue et al. (1995)	Jin et al.(2000)		Portyanko et al.(2001)	Groh et al.(2001a)		Zhu and Kaeppler(2003a)	Wight et al. (2003)
	'Kanota' x 'Ogle'	'Kanota' x 'Ogle'	Clintland64 x IL86-5698	'Ogle' x TAM O-301	'Kanota' x 'Ogle'	'Kanota' x 'Marion'	'Ogle' x MAM17-5	'Kanota' x 'Ogle'
RFLP	537	228		355	660	60	172	748
AFLP	0	263	265	40	86	118	324	371
RAPD	7	0		22			0	22
SSR	0	0		1			14	6
STS	3	0		7			0	2
other markers	14			16			0	17
Total	561	491	265	441	756	178	510	1166
Linkage groups	38	28	30	34	34	27	28	29
Unlinked loci	29	NR	44	15	15	33	34	43
Length of map (cM)	1482	2351	1363	2049	NR	736	1397	1890

The first QTL analysis for oat *β*-glucan content was conducted by Kianian et al. (2000). In their study, two recombinant inbred populations sharing a common parent were used for QTL analysis. Regions influencing *β*-glucan content on linkage groups 11 and 14 of the oat KO RFLP map were identified in both populations and over environments.

3.6 Oat powdery-mildew resistance

Powdery-mildew is one of the most common plant diseases, infecting over 650 monocot and over 9000 dicot species. Caused by *Erysiphe graminis* D.C.f. sp. *avenae* Em. Marchal, powdery-mildew is an important foliar disease of cultivated oat worldwide, especially in humid regions such as maritime northwest Europe and along the Atlantic seaboard. In Great Britain, it is the most damaging fungal disease of oat and can decrease grain yield by up to 39% (Lawes and Hayes 1965). Hsam et al. (1997) investigated powdery-mildew resistance in 259 common oat cultivars and breeding lines. They found 67% of the investigated plants were susceptible to this disease.

For economical and environmental reasons, the breeding of resistant varieties would be the most feasible means of controlling this disease and reducing yield losses.

3.6.1 Resources of oat powdery-mildew resistances

According to the disease reaction characteristics of standard cultivars and lines to different powdery-mildew isolates, resistance has been categorised as oat mildew resistance (OMR) groups (Table 3.2, from Roderick et al., 2000). The resistance is a kind of immune reaction based on major gene differences in reaction to specific pathogen races. It is expressed at the seedling stage and normally the resistance can be maintained throughout all stages of plant's growth. Several sources with major gene resistance to powdery-mildew, including common oats (Jones 1983; Hsam et al. 1997; Hsam and Zeller 1998), wild oat species such as *A. barbata* (Aung et al. 1977; Thomas et al. 1980), *A. strigosa*, *A. occidentalis* (Herrmann and Roderick 1996), *A. pilosa* (Hoppe and Kummer 1991) and *A. sterilis* (Hayes and Jones 1966), have been reported. Hsam et al. (1997) reported that resistant cultivars with documented oat mildew resistance (OMR) are mainly grown in France, Germany and Great Britain. And the

more common resistance groups present in cultivars are OMR groups 2 and 3.

Oat adult-plant resistance (APR) is a more durable form of resistance which expresses itself most strongly in post-seeding stages of growth and is better expressed under field conditions. Adult-plant resistance is different from the immune reaction at the seedling stage of OMR groups. It can't be affected by differences in pathogenicity of the fungus. It is said that for long-term solution of the control of disease, more attention should be paid to adult-plant resistance than that of single genes reactions which may loose their functions because of the changes of pathogenicity of the mildew. Oat APR has remained effective for many years in some UK varieties, such as 'Maldwyn' which was introduced in the 1940s with moderate APR (Joes, 1978).

Two main sources of powdery-mildew resistance are currently deployed in European oat breeding programs. The first source of powdery-mildew resistance is from that of common oat line Pc54. The line was originally derived from a cross between *A. sativa* and *A. sterilis* (Sebesta et al., 1993). The powdery-mildew resistance of line Pc54 is controlled by a single incompletely dominant gene. It is not fully expressed at the seedling stage and can be described as adult-plant resistance. The second source is from lines APR122 and APR166. The lines were initially derived from a hybrid between *A. eriantha* and *A. sativa*, described as adult-plant resistance and controlled by a dominant gene which was incompletely expressed at the seedling stage (Hoppe and Kummer, 1991). However, Hsam et al. (1997) reported APR122 also showed full resistance at the seedling stage to all the differential isolates used in their study. These lines are the most promising source of resistance to oat powdery-mildew.

Though lots of resistance sources have been characterized and deployed in oat breeding programmes, it is suggested to investigate new stable sources of resistance and perform combinations of available resistance genes, which will provide more comprehensive protection from the infections of powdery-mildew.

Table 3.2 Oat mildew resistance (OMR) groups (Roderick et al., 2000)

OMR group	Differential cultivar	Gene Designation	Source of resistance
0	Milford		
1	Manod		*A. byzantina* variety Red Algerian
2	Cc4146	*Eg-1*	*A. sativa* x *A. ludoviciana*
3	9065Cn 6/3/74	*Eg-3*	*A. ludoviciana* (Cc4346)
4	Cc6490	*Eg-4*	*A. barbata* (Cc4897)

3.6.2 *Avena macrostachya*, a useful wild species for oat breeding

Avena macrostachya is the only perennial and cross-fertilizing wild oat species within the genus *Avena*. It is a tetraploid species while its exact genome constitution is still not clear. *A. macrostachya* possesses several traits with high relevance for oat breeding. Beside the winter hardiness (Baum and Rajhathy, 1976), the resistances to Barley Yellow Dwarf Virus (BYDV), *Erysiphe graminis* (Hoppe and Pohler 1988) and *Rhopalosiphum padi* (Weibull, 1986) have also been reported in *A. macrostachya*. Interspecies crosses with *A. macrostachya* are quite difficult, which limited the application of this wild species for cultivated oat breeding. Several crosses of *A. macrostachya* with wild and cultivated oat species were released to study the genome constitution and to transfer valuable traits into *A. sativa* (Pohler and Hoppe, 1991; Leggett, 1985). However, there is no comprehensive report about a successful introgression of genes from *A. macrostachya* to *A. sativa* up to now.

3.6.3 Application of molecular markers for disease resistance in oat

DNA-based molecular markers have greatly increased the capacity for more precise prediction of a genotype in plant breeding. Currently the major molecular markers used for oat mapping are RFLPs and AFLPs. However, for high-throughout genotyping, PCR-based markers are highly desired by plant breeders. As PCR-based markers, SSR markers are particularly important for that they are codominant, locus-specific, and evenly distributed along chromosomes. High-density SSR maps have been developed in several important crops including wheat (Röder et al., 1998) and barley (Ramsay et al., 2000). In oat, only very limited SSR sequences have been reported by Li et al. (2000), Holland et al. (2001), and Pal et al. (2002). Despite the limited information of SSR markers, conversion of AFLP markers to

sequence-tagged-site (STS) PCR markers would be beneficial for oat Marker Assisted Selection (MAS) breeding.

A number of markers that are tightly linked to resistance genes have been widely applied for genetic analysis and breeding purpose in different crop species. In cultivated oats, Penner et al. (1993a) were the first to identify a molecular marker for a stem-rust resistance gene. To date, a number of different markers linked to genes or QTLs involved in oat resistance reactions against stem rust (O'Donoughue et al., 1996; Cheng et al., 2002), crown rust (Penner et al., 1993b; Rooney et al., 1994; Bush and Wise, 1998; Zhu and Kaeppler, 2003b; Chong et al., 2004; Wight et al., 2004), and BYDV (Zhu et al., 2003), have also been identified.

For oat powdery-mildew resistances, however, no linked molecular markers have been developed till now. Therefore, developing molecular markers tightly linked to the powdery-mildew resistance genes would be beneficial both for oat breeding purposes and for the investigation of oat genomic regions containing interesting resistance genes.

4 Materials and Methods

4.1 AB-QTL analysis

4.1.1 Population development

Four non-adapted high-*β*-glucan lines ('Otee', 'Ariane', IAH611-447, PA 82 90; supported by Dr. H.E. Bockelman, USDA) were crossed with four high-yielding cultivars ('Vital', 'Iltis', 'Nordstern', 'Expander'; supported by Nordsaat Saatzucht GmbH) with different *β*-glucan levels. From obtained 16 crosses F_1, BC_1 and BC_2 were produced. Evaluation of *β*-glucan contents was performed in BC_1F_2 in two different locations, Granskevitz and Groß Lüsewitz. Two BC_1F_2 progenies derived from the cross of 'Iltis' x IAH611-447 were found with the highest *β*-glucan content. Subsequently, three BC_2F_2 sub-populations traceing back to the two highest *β*-glucan BC_1 plants were developed. There were a total of 237 BC_2F_2 derived lines with 98 lines in sub-population 2503, 72 lines in sub-population 2504, and 67 lines in sub-population 2803, respectively (Figure 4.1).

Due to limited sources at the beginning of this programme, no DNA of the F_2 plants from 'Iltis' x IAH611-447 was isolated. And because of the rather low number of BC_1 individuals used for backcross and a low number of available BC_2 individuals, it was decided to use the segregating BC_2F_2 sub-population 2503 which is derived from a single BC_2 individual for both mapping and QTL analysis.

4.1.2 Field evaluations

For field testing, each BC_2F_2 individual was bulk propagated until $BC_2F_{2:6}$ (2003) and $BC_2F_{2:7}$ (2004). The backcross lines were grown at the three locations Böhnshausen (Saxony-Anhalt, Central Germany), Granskevitz (Isle of Rügen, North-East Germany), and Gudow (Schleswig-Holstein, North Germany) in two years, 2003 and 2004, respectively (Table 4.4). The lines were grown in 10-row plots with a 12.5-cm row spacing, a seedling rate of 350 seeds m^{-2} and a harvest of 3.75m^2. All three sub-populations together with 7 cultivars and the parents of the populations were arranged in a randomised complete block design with two replications at each environment.

Most important ecological data of the environments are summarised in Table 4.1 and Table 4.2. At all stations the soil types are sandy loam, but among the 3 stations the deepest topsoil is measured in Böhnshausen and the lowest in Gudow. Fertilizer was given according to local practice and all experiments were kept free of weeds.

Table 4.1 Soil type and geographic characteristics of the locations

Location	Geographic characteristic			
	Type of soil	Soil value points	Latitude/longitude	Altitude
Böhnshausen	sandy loam	74	51°30´ / 11°01´	202m
Granskevitz	sandy loam	54	54º45´ / 13°25´	3m
Gudow	sandy loam	50	53°57´ / 10°70´	35m

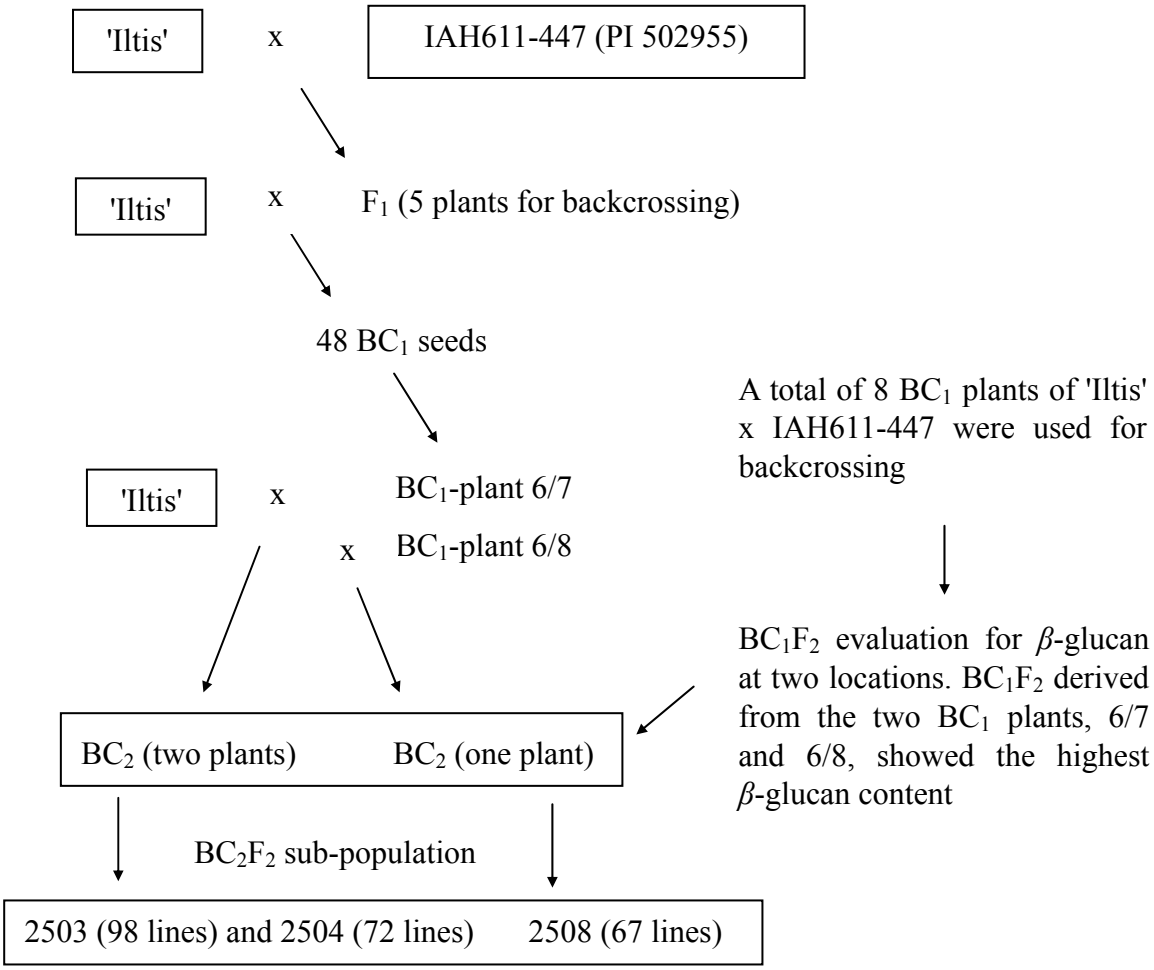

Figure 4.1 Scheme of the population development for the AB-QTL-programme. 'Iltis' is high yielding oat cultivar; IAH611-447 is a non-adapted oat line with high *β*-glucan

Table 4.2 Precipitation (litre per m^2) of the 3 locations in two years

Month	2003			2004		
	Böhnsh.	Gransk.[1]	Gudow[2]	Böhnsh.	Gransk.[1]	Gudow[2]
June	33	26-50	26-50	74	51-75	76-100
July	49	26-50	26-50	65	76-100	76-100
August	22	26-50	26-50	51	76-100	51-75

[1]Source: measurements from Arkona station of DWD: no mean values available

http://www.dwd.de/de/FundE/Klima/KLIS/daten/online/klimakarten/showmap.htm

[2] Source: Grambek station of DWD, no mean values available

4.1.3 Trait evaluations

A total of 11 agronomic traits were evaluated for each plot in 2 to 6 environments as described in Table 4.3. Particularly, groat β-glucan content was evaluated using two methods, NIRS and enzymatic assay, respectively.

Table 4.3 List of 11 traits measured in up to six environments (3 locations in 2 years)

Trait	Abbreviation	Method of measurement
Yield (dt per ha)	YLD	Plot yield, weighed after harvesting with a combine and purifying with a stationary threshing device
Heading	HEA	Number of days from the 1st January to panicle emergence
Height	HCM	Plant height
Panicle number per m^2	PAN	Number of panicles per m^2 at maturity
Powdery-mildew infection	PMI	Visual assessment of each plot from 1= no infection to 9= severe infection
Hull content	HUC	Percent hull after dehulling of 100g sample with an compressed air dehuller.
Thousand-grain weight	TKW	Mass of 1000 kernels, deduced from a counted 15g-sample after cleaning with an air separator
Test weight	HLW	Test weight is a measure of the density of oat grains as they are packed into a given volume
Protein content	PRO	Protein content, measured with NIRS of ground oat; % of dry matter
Groat lipid	LIP	NIRS measurement of crude fat content of groats; % of dry matter
Groat β-glucan content	BG(N)	NIRS (near infrared reflectance spectroscopy) measurement of beta glucan content; % of dry matter
	BG(E)	Enzymatic assay according to McCleary and Glennie-Holmes (1985)

Table 4.4 Number of replication for trait evaluation of the AB-QTL population in 2003 and 2004 at three locations

	Environment[b]					
Trait[a]	Böh03	Gra03	Gud03	Böh04	Gra04	Gud04
YLD	2	2	2	2	2	2
HEA	2	2	2	2	2	2
HCM	2	2	2	2	2	2
PAN	-	2	2	2	2	2
PMI	2	2	-	2	2	2
HUC	1	1	1	1	1	1
TKW	1	1	1	1	1	1
HLW	1	1	1	1	1	1
PRO	1	1	1	1	1	1
LIP	1	1	1	1	1	1
BG(N)	1	1	1	-	-	-
BG(E)	-	1	-	-	1	-

[a]Abbreviation of traits according to table 4.3
[b] Coded by location, where Böh, Böhnshausen; Gra, Granskevitz; Gud, Gudow; followed by year

4.1.4 DNA isolation

Leaf material was collected from young BC_2F_2 plants. Genomic DNA was isolated from frozen leaves using modified CTAB procedure (Saghai-Maroof et al., 1984).

4.1.5 SSR analysis

144 oat SSR primer pairs, of which 61 were from Li et al. (2000), 44 were from Pal et al. (2002), 9 were from Holland et al. (2001) and 30 were derived from oat EST data, were used for screening of polymorphisms between the two parents of the mapping population and two bulks. For each forward primer, an additional M13 tail was added. The PCR amplifications were performed in 10 µL reaction mixes containing 20 ng of template DNA; 0.5 pmol of each forward primer, 5 pmol of each reverse primer, and 5 pmol of M13 primer labelled with an infrared fluorescent dye (IRD 700); 0.25 U of HotStar Taq DNA polymerase and 1x HotStar Taq PCR buffer (QIAGENE GmbH, Hilden, Germany); 0.2 mM of dNTPs. The PCR reaction

was carried out on a Peltier Thermal Cycler PTC-200 (MJ Research, Waltham MA, USA) using a "Touchdown" PCR profile: started with an initial denaturation at 95°C for 15 min; followed by 20 cycles of 1 min at 94°C, 1 min at 65°C, 1 min at 72°C. Annealing temperatures were progressively decreased by 0.5°C each cycle to 55°C. PCR continued for 23 additional cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C with a final elongation step of 72°C for 10 min. PCR products were separated on an automated laser fluorescence (ALF) sequencer (Pharmacia Biotech) using a KB^{Plus} 6.5% gel matrix (LI-COR GmbH, Bad Homburg, Germany) at 1500 V, 60 mA, 30 W and 50°C. Fragment sizes were calculated using the Fragment Analyser version 1.00 program (Pharmacia Biotech) by comparison with the external size standards.

4.1.6 AFLP analysis

AFLP analysis was conducted as described by Vos et al. (1995) with modifications. A total of 500 ng genomic DNA was digested with *Eco*RI and *Mse*I. *Eco*RI and *Mse*I adapters were ligated to the restriction fragments. The ligation mixture was 1:10 diluted. Preselective amplification was performed using primers with one additional nucleotide (*Eco*RI+A, *Mse*I+C). Selective amplification was performed using primers with three additional

Table 4.5 Sequences of AFLP primers used for selective amplifications

Primers	*Eco*RI core sequence[a]	Primers	*Mse*I core sequence[b]
E31	-AAA	M47	-CAA
E32	-AAC	M48	-CAC
E33	-AAG	M49	-CAG
E34	-AAT	M50	-CAT
E35	-ACA	M51	-CCA
E36	-ACC	M52	-CCC
E37	-ACG	M53	-CCG
E38	-ACT	M54	-CCT
E39	-AGA	M55	-CGA
E40	-AGC	M56	-CGC
E41	-AGG	M57	-CGG
E42	-AGT	M58	-CGT
E43	-ATA	M59	-CTA
E44	-ATC	M60	-CTC
E45	-ATG	M61	-CTG
E46	-ATT	M62	-CTT

[a] *Eco*RI primer core sequences: 5'-GACTGCGTACCAATTC-3'
[b] *Mse*I primer core sequences: 5'-GATGAGTCCTGAGTAA-3'

nucleotides. Each of the *Eco*RI selective primers was labelled with an infrared fluorescent dye (IRD 700 or IRD 800). A total of 256 primer pair combinations were employed for screening (Table 4.5). The selective amplification products were detected on a LI-COR automated sequencer (LI-COR, Lincoln, Nebraska) using a KB^{Plus} 6.5% gel matrix (LI-COR GmbH, Bad Homburg, Germany) at 1500 V, 40 mA, 25 W and 45°C. The AFLP loci were named based on the primer combination followed by a number which referred to the specific polymorphisms.

4.1.7 Statistical analysis

Trait data analysis

Each year-location combination was considered as one environment (Table 4.4). Mean values across replications were calculated over environments. Mean values for each trait for each environment were also calculated with Student-Newman-Keuls Test by SAS 6.12 GLM procedure (SAS Institute, Cary, NC, USA). Distribution for each trait across environments and correlations between environments for each trait were performed using the computer program PLABSTAT (Utz 2000). Correlations between traits were calculated separately for each environment for all traits. Analysis of variance was performed by SAS 6.12, and the F value of ANOVA for genotype, environment, and genotype x environment interaction were calculated.

Evaluation of agronomic values

A selection index was calculated for each line and cultivar using the results from the field evaluations. Using the relative mean value of each line over all environments, each trait was weighted and the weighting factor was multipled with appropriate relative values and the trait heritability. The index consisted of all product values (products of weighting factor, relative value and the heritability) while a high index value was aimed for.

Index= Σ (A) - Σ (B)

A= (relative value*weighting factor) of YLD, TKW, PRO, BG(N), BG(E)

B= (relative value*weighting factor) of HEA, HCM, PMI, HUC, LIP

Weighting factor

YLD	TKW	PRO	BG(N)	BG(E)	HEA	HCM	PMI	HUC	LIP
5	3	2	3	3	2	2	1	3	1

Map development

Linkage maps were constructed with the JoinMap 3.0 program (Van Ooijen and Voorrips, 2001). A LOD score of 3.0 was established to consider significant linkage, and the Kosambi mapping function was used to convert recombination fractions into centiMorgans (cM).

QTL analysis

11 traits (Table 4.3) were analyzed for QTL detection. QTL analysis was performed using computer program PlabQTL (Utz and Melchinger, 1996). Simple interval mapping (SIM) was used to identify QTL regions. A QTL was confirmed when the LOD score exceeded the threshold of 2.5 over two environments or with a LOD exceeding 3.0 in one environment. QTLs with an overlapping support interval are assumed to be the same QTL.

4.2 Genetic analysis of powdery-mildew resistance

4.2.1 Plant materials

Interspecific crosses between resistant *A. macrostachya* with susceptible *A. barbata*, *A. magna* and *A. sativa*, respectively, were developed by Pohler and Hoppe (1991). One specific cross ((*A. magna* x *A. macrostachya*) x AS93) x AS93, realised via embryo rescue, was handed over for the present study. This cross was further used for the development of stable introgression lines with powdery-mildew resistance from *A. macrostachya* as shown in Figure 4.2.

To investigate the inheritance of powdery-mildew resistance introgressed from *A. macrostachya*, resistant BC_2F_7 line Am27 and BC_2F_6 line Am28 were crossed with susceptible cultivars 'Neklan' and 'Flämingsprofi' for the development of segregating populations. F_2, F_3 and BC_1 populations of all crosses were tested for segregation analysis of powdery-mildew resistance using detached-leaf segment tests.

For linkage analysis, 180 randomly selected F_3 families from the cross of resistant line Am28 with susceptible cultivar 'Flämingsprofi', and 144 selected F_3 families from the cross of resistant line Am27 with susceptible cultivar 'Neklan' were used. For comparative mapping, 71 F_{10} recombinant inbred lines of the 'Kanota' x 'Ogle' mapping population (Wight et al.,

2003), kindly provided by Dr. Nicholas A. Tinker (Agriculture and Agri-Food Canada), were used.

((*A. magna* x *A. macrostachya*) x AS93) x AS93 (Pohler and Hoppe 1991)

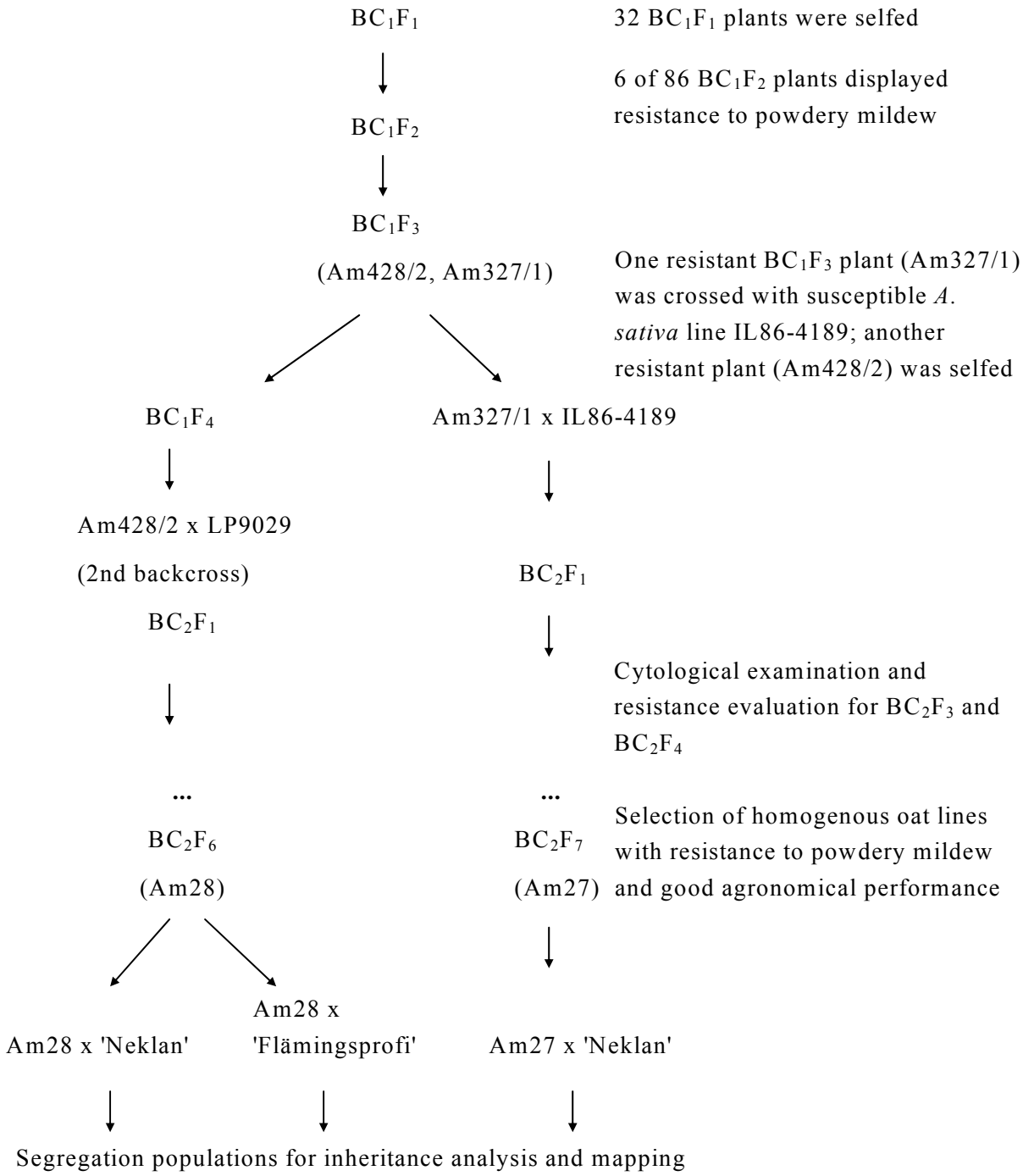

Figure 4.2 Development of progenies with powdery-mildew resistance derived from *A. macrostachya* CAV 5264. AS93 is an *A. sativa* line with good crossing ability. Cytological examinations were performed as described by Linares et al. (1996).

4.2.2 Resistance testing

Leaf segments about 2 cm in length from seedlings grown in a glasshouse were placed on the surface of benzimidazole agar (0.6% agar, 30ppm benzimidazole) in clear rectangular polystyrene boxes. Inoculation was performed using an infection tower with an interchangeable, 5-bar air-pressured plexiglass device. A mixture of powdery-mildew collections which had been propagated on leaf segments of a susceptible oat line was used for inoculation. Freshly harvested spores were dispersed over the leaf segments with 200-800 spores per cm^2. For a reliable classification of individuals, the resistant and susceptible parental genotypes were also included in each test box. Following 8-10 days incubation in a growth chamber at 18-20°C, 16-h daylength with 4 kLx fluorescent lighting (Philips TLD 58W/25), reaction of leaf segments to powdery-mildew was scored using a 0-5 scale (Table 4.6). For segregation analysis, leaf segments with scores 0, 1 or 2 were grouped as resistant while scores 3, 4, and 5 were summarised as susceptible. In F_2 and BC_1 examinations the seed leaves and the first- and second-true leaves were used in subsequent tests. For F_3 generation, 15 plants per family were examined.

Table 4.6 Description of symptoms and scoring of oat powdery-mildew resistance in leaf-segment tests

Score	Symptoms
0	no symptoms of infection
1	sparse mycelium, no sporulation
2	weak sporulation, mycelium cover less than 10% of segment surface area
3	moderate sporulation, mycelium cover about 30% of segment surface area
4	abundant sporulation, mycelium cover 30-80% of segment surface area
5	abundant sporulation, mycelium cover more than 80% of segment surface area

4.2.3 DNA isolation and bulked segregant analysis

Total genomic DNA was extracted from approximately 1g of young leaf tissue using DNeasy Plant Maxi Kits (QIAGENE GmbH, Hilden, Germany), following the manufacturer's instructions. For SSR bulked-segregant analysis (Michelmore et al. 1991), two DNA bulks were established by using equal amounts of DNA from twelve homozygous resistant and twelve homozygous susceptible F_3 families, respectively. For AFLP bulked-segregant analysis,

two bulks were made by using equal amounts of pre-amplified DNA obtained from the same twelve homozygous resistant and twelve homozygous susceptible F_3 families, respectively.

4.2.4 SSR and AFLP analysis

SSR and AFLP analysis were the same as 4.1.5 and 4.1.6, respectively.

4.2.5 Cloning and conversion of AFLP markers

In order to isolate linked AFLP fragments, unlabelled AFLP selective primers were used instead of labelled primers. PCR products were separated on 6% polyacrylamide denaturing sequencing gels. Gels were run at 50W constant power, silver stained, and dried. Appropriate AFLP fragments were excised from the dried gel. The bands were eluted from the gel by incubation in 50 µL of TE buffer at 4°C overnight. 2 µL of TE buffer containing eluted bands were used for re-amplification using the same AFLP selective primers with the same PCR protocols. Amplified products with correct size were recovered from agrose gel using the Qiaquick gel extraction kit (QIAGENE GmbH, Hilden, Germany). Recovered DNA fragments were cloned using the pGEM-T vector system (Promega GmbH, Mannheim, Germany), following the manufacturer's instructions.

For dominant AFLP markers, the band representing the dominant allele was cloned. For codominant AFLP markers, the bands for both alleles were cloned. For each recovered DNA band, 2 clones which contain the target fragments confirmed by PCR with corresponding AFLP selective primer pairs were selected for sequencing. New primer pairs were designed based on sequences information of clones. The new primer pairs that revealed polymorphism between the two parents were further used to screen the mapping population.

4.2.6 Linkage analysis

Linkage analysis was performed with the JoinMap 3.0 program (Van Ooijen and Voorrips 2001). A LOD score of 3.0 was established to consider significant linkage, and the Kosambi mapping function was used to convert recombination fractions into centiMorgans (cM).

5 Results

5.1 AB-QTL analysis

5.1.1 Phenotypic traits

The correlation coefficients for each trait between different environments were mostly positive and significant (Table 5.1), except for the traits of panicle number and powdery-mildew infection. For panicle number, significant correlations were found between Gra03 and Gud04, Gud03 and Gra04, Gra04 and Gud04. No significant correlations were detected between other environments. For powdery-mildew infection, Böh04 was not significantly correlated with any other environments.

All the 11 traits evaluated for the sub-population 2503 across environments showed a pattern of continuous distribution around the mean, although some traits did not show a perfect normal distribution (Figure 5.1). The phenotypic distributions of the $BC_2F_{2:6-7}$ population agree that the traits evaluated are quantitative traits.

The mean values across environments of the 11 traits for both parents and the sub-population 2503 are given in Table 5.2, together with the range and the skewness of the population for each trait. For yield, height of the plot, panicle number, powdery-mildew infection and protein content, significant skewness to the recurrent parent 'Iltis' (P2) was found. For β-glucan content, no significant skewness was found for the values obtained using the NIRS measurement method. However, significant skewness to the recurrent parent 'Iltis' was found when the β-glucan content was measured using the method of enzymatic assay (Table 5.2)

Table 5.1 Pearson's correlation coefficients of 11 traits between environments

YLD

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.516**				
Gud03	0.533**	0.500**			
Gud04	0.686**	0.645**	0.524**		
Böh03	0.581**	0.522**	0.485**	0.481**	
Böh04	0.354**	0.299**	0.348**	0.179	0.353**

HCM

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.838**				
Gud03	0.516**	0.533**			
Gud04	0.788**	0.814**	0.534**		
Böh03	0.739**	0.769**	0.489**	0.662**	
Böh04	0.736**	0.835**	0.520**	0.710**	0.728**

HUC

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.456**				
Gud03	0.607**	0.260*			
Gud04	0.550**	0.255*	0.372**		
Böh03	0.380**	0.404**	0.323**	0.405**	
Böh04	0.463**	0.061	0.407**	0.414**	0.365**

HLW

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.552**				
Gud03	0.661**	0.565**			
Gud04	0.549**	0.443**	0.562**		
Böh03	0.507**	0.132	0.452**	0.343**	
Böh04	0.518**	0.514**	0.669**	0.423**	0.213*

LIP

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.372**				
Gud03	0.605**	0.564**			
Gud04	0.189	0.349**	0.288**		
Böh03	0.471**	0.523**	0.804**	0.311**	
Böh04	0.305**	0.437**	0.580**	0.186	0.552**

HEA

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.862**				
Gud03	0.895**	0.889**			
Gud04	0.840**	0.924**	0.888**		
Böh03	0.919**	0.833**	0.859**	0.827**	
Böh04	0.737**	0.837**	0.853**	0.881**	0.686**

PAN

	Gra03	Gra04	Gud03	Gud04
Gra04	0.129			
Gud03	0.095	0.215*		
Gud04	0.400**	0.188	0.246*	
Böh04	0.095	0.02	0.075	-0.054

PMI

	Gra03	Gra04	Gud04	Böh03
Gra04	0.312**			
Gud04	0.595**	0.391**		
Böh03	0.409**	0.435**	0.578**	
Böh04	-0.136	0.083	0.032	-0.05

TKW

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.378**				
Gud03	0.162	0.432**			
Gud04	0.480**	0.621**	0.421**		
Böh03	0.322**	0.409**	0.361**	0.431**	
Böh04	0.386**	0.584**	0.470**	0.682**	0.386**

PRO

	Gra03	Gra04	Gud03	Gud04	Böh03
Gra04	0.569**				
Gud03	0.537**	0.353**			
Gud04	0.423**	0.474**	0.409**		
Böh03	0.594**	0.545**	0.352**	0.471**	
Böh04	0.290**	0.297**	0.261**	0.195	-0.002

BG(N)

	Gra03	Gud03
Gud03	0.543**	
Böh03	0.478**	0.394**

BG(E)

	Gra03
Gra04	0.288**

*, ** Significant at P =0.05 and P =0.01, respectively

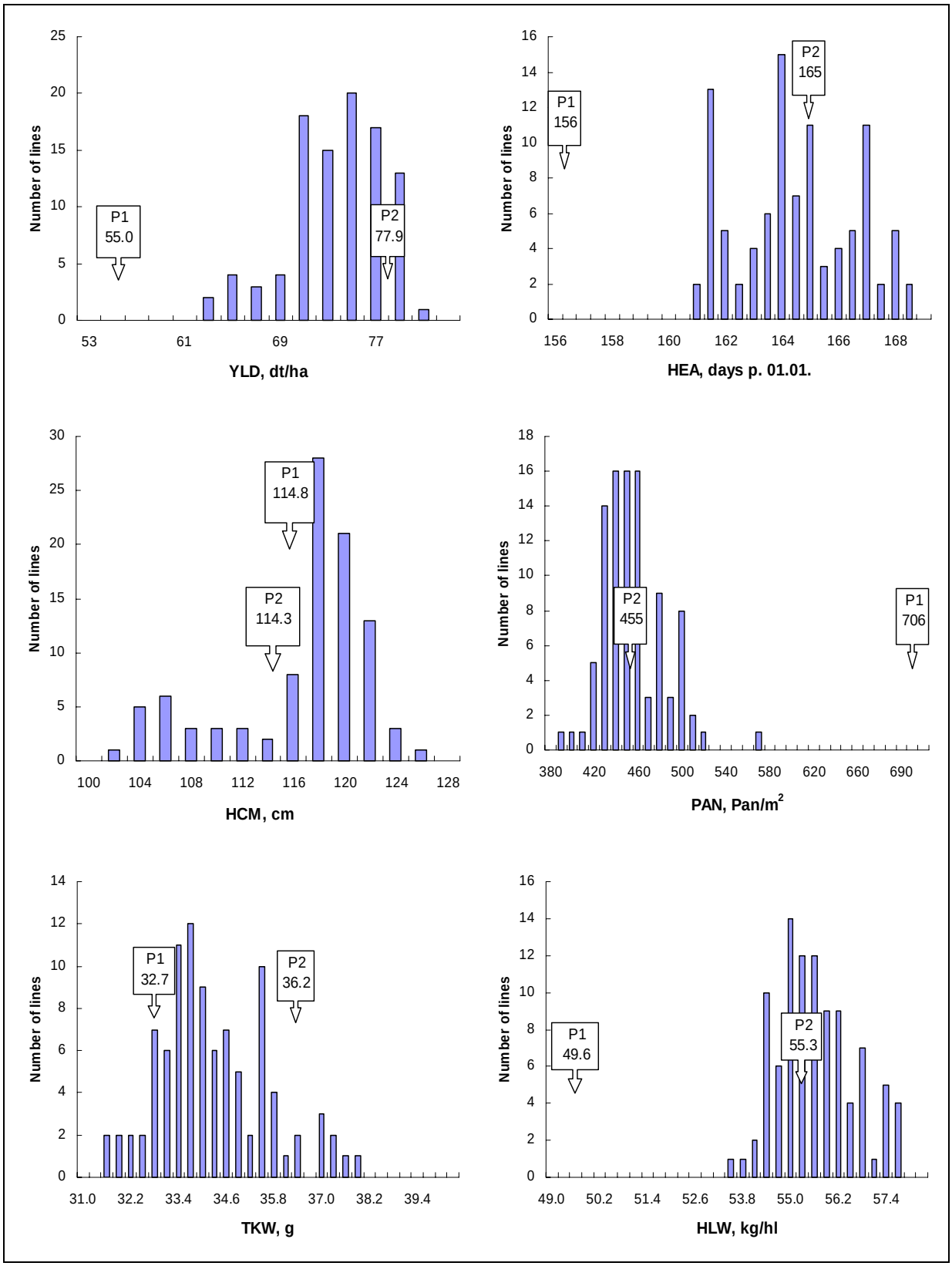

Figure 5.1 Phenotypic distributions of the 11 traits of the sub-population 2503 using the mean values across all environments. P1: IAH611-447; P2: 'Iltis'

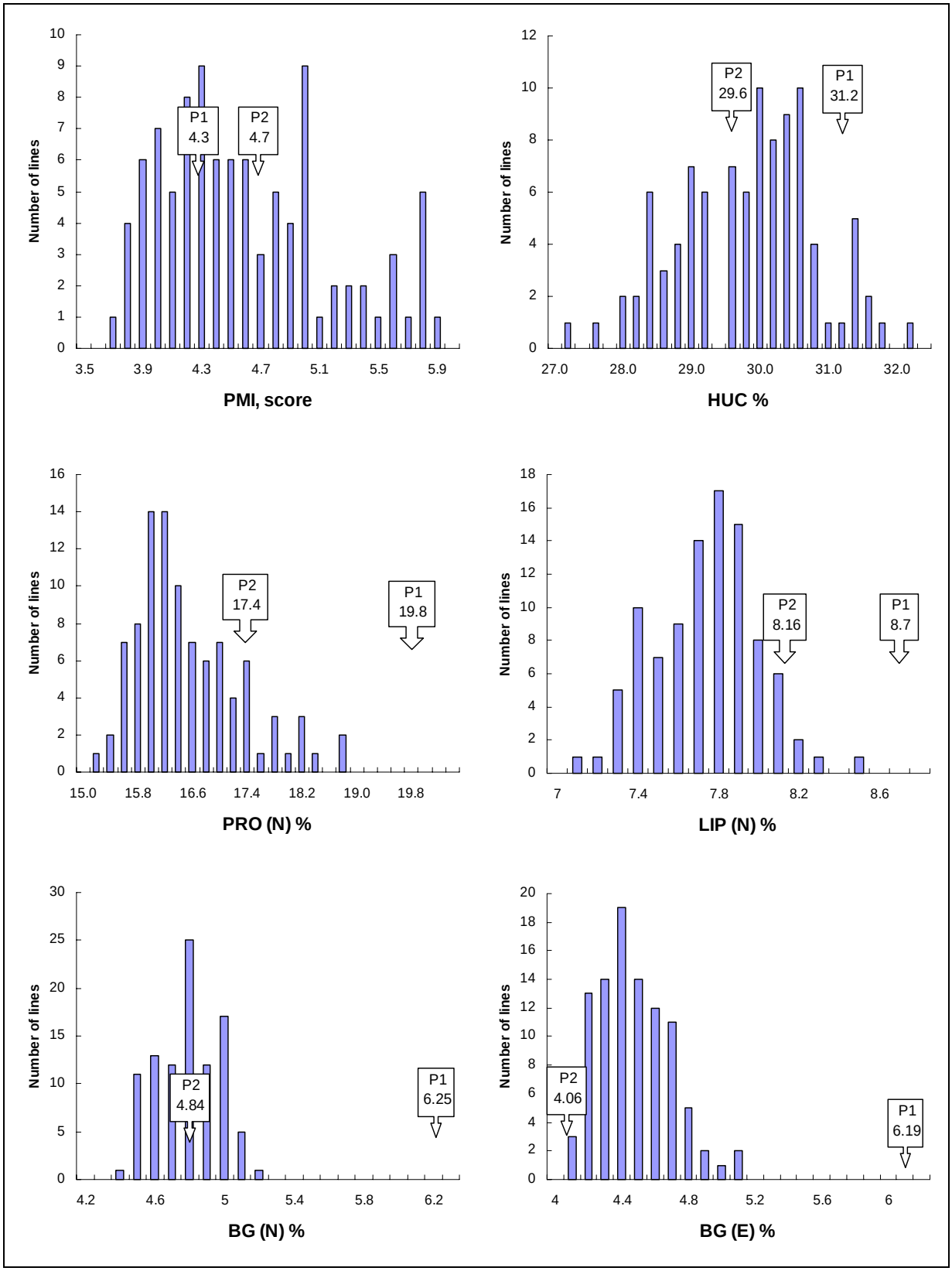

Figure 5.1 (Continued) Phenotypic distributions of the 11 traits of the sub-population 2503 using the mean values across all environments. P1: IAH611-447; P2: 'Iltis'

Table 5.2 Mean value of 11 traits for both parents and the sub-population 2503

Traits	Parents[a]		Population			
	P1	P2	Mean	Minimum	Maximum	Skewness
Yield	45.0	77.9	72.8	61.1	79.7	-0.78**
Heading	156.1	164.9	163.8	160.0	168.0	0.16
Height of the plot	114.8	114.3	115.7	102.0	125.0	-1.09**
Panicle number	706.4	455.2	450.9	385.0	568.0	0.95**
Powdery-mildew infection	4.3	4.7	4.6	3.7	5.9	0.60*
Hull content	31.2	29.6	29.8	27.2	32.1	-0.2
Thousand-grain weight	32.7	36.2	34.1	31.4	37.7	0.47
Test weight	49.6	55.3	55.5	53.4	57.7	0.41
Protein content	19.8	17.4	16.4	15.1	18.7	0.89**
Lipid content	8.7	8.2	7.7	7.1	8.4	-0.08
β-glucan content (N)	6.3	4.8	4.7	4.4	5.2	0.06
β-glucan content (E)	6.2	4.1	4.4	4.1	5.1	0.58*

a: P1: IAH611-447; P2: 'Iltis'
*, ** Significant at P =0.05 and P =0.01, respectively

5.1.2 ANOVA for genotypes and environments

The analysis of variance for the 11 traits is presented in Table 5.3. The results indicate significant differences between environments and among population lines for all the traits evaluated. For those traits which had replication trials, significant genotype x environment interactions were found for heading date (P<0.01) and plant height (P<0.01).

The mean values of the 11 traits for each environment are shown in Table 5.4. The highest yield, combined with the highest plant height and panicle numbers was found for Böhnshausen 2004. The highest value of β-glucan (BG(N)) content was found for Böhnshausen 2003. Compared with the BG(E) means of both years, a higher value was found in 2003, in which year there is a lower precipitation (Table 5.2).

Table 5.3 The F value of ANOVA for genotypes (G), environments (E) and genotype x environment interaction (GE) of the 11 traits

YLD		
Source	DF	F
E	5	249.78**
G	96	6.00**
GE	480	1.02

HEA		
Source	DF	F
E	5	5787.09**
G	96	32.96**
GE	480	2.14**

HCM		
Source	DF	F
E	5	1815.37**
G	96	13.46**
GE	480	1.56**

PAN		
Source	DF	F
E	4	126.00**
G	96	1.76**
GE	384	0.89

HUC		
Source	DF	F
E	5	214.52**
G	96	3.59**
GE	480	

TKW		
Source	DF	F
E	5	148.79**
G	96	5.32**
GE	480	

PMI		
Source	DF	F
E	3	607.49**
G	96	4.00**
GE	288	1.18

HLW		
Source	DF	F
E	5	143.00**
G	96	5.11**
GE	480	

PRO		
Source	DF	F
E	5	158.83**
G	96	4.40**
GE	480	

LIP		
Source	DF	F
E	5	46.52**
G	96	4.94**
GE	480	

BG(N)		
Source	DF	F
E	2	200.76**
G	96	3.63**
GE	192	

BG(E)		
Source	DF	F
E	1	215.32**
G	96	1.62**
GE	96	

*, ** Significant at $P = 0.05$ and $P = 0.01$, respectively

Table 5.4 Mean value of the 11 traits for each environment with Student-Newman-Keuls Test (SNK) Test

Trait	Environments					
	Gra03	Gra04	Gud03	Gud04	Böh03	Böh04
Yield	66.00^{D}	71.08^{B}	71.21^{B}	67.66^{C}	79.84^{A}	81.06^{A}
Heading	160.04^{D}	167.71^{B}	161.21^{C}	167.70^{B}	158.24^{E}	169.32^{A}
Height of the plot	99.59^{F}	122.53^{B}	106.79^{E}	111.01^{D}	117.38^{C}	136.57^{A}
Panicle number	364.25^{D}	476.29^{B}	452.45^{C}	437.98^{C}	-	523.42^{A}
Powdery-mildew infection	5.88^{A}	2.60^{D}	-	5.96^{A}	5.37^{B}	3.22^{C}
Hull content	29.14^{C}	27.18^{D}	33.12^{A}	29.17^{C}	29.77^{B}	30.10^{B}
Thousand-grain weight	35.26^{A}	35.09^{A}	31.09^{C}	35.62^{A}	35.03^{A}	32.73^{B}
Test weight	55.25^{B}	57.81^{A}	55.60^{B}	54.33^{C}	54.27^{C}	55.66^{B}
Protein content	17.28^{B}	15.24^{E}	14.96^{E}	16.44^{D}	17.86^{A}	16.90^{C}
Lipid content	7.45^{E}	7.61^{D}	7.96^{A}	7.52^{DE}	7.73^{C}	7.84^{B}
β-glucan content (N)	4.66^{B}	-	4.57^{C}	-	5.01^{A}	-
β-glucan content (E)	4.69^{A}	4.17^{B}	-	-	-	-

Mean values with different superscript letters are significantly different at $P = 0.05$

5.1.3 Correlation between traits

Significant correlation coefficients were found between all the 10 traits analyzed in most environments (Table 5.5). For yield, high positive correlations were found with heading date, height of the plot and groat lipid content in 5 of all 6 environments, and with hull content in 3 of 6 environments; negative correlations were found with panicle number, powdery-mildew infection, thousand-grain weight, test weight and groat protein content in most environments. Negative correlation between groat protein content and lipid content were found in 3 of 6 environments, however, in Gud04, positive correlation was found between these two traits.

Correlation coefficients between β-glucan content and all other traits were calculated separately for each environment and measurement method (Table 5.6). Significant negative correlations were found between β-glucan content and yield, heading date, height of the plot for all environments, and hull content, groat lipid content for 3 environments. Significant positive correlations were found between β-glucan content and groat protein content, panicle number, powdery-mildew infection for all environments, and thousand-grain weight and test weight for 2 and 3 environments, respectively.

Table 5.5 Pearson's correlation coefficients between 10 traits within each environments

	YLD	HEA	HCM	PAN	PMI	HUC	TKW	HLW	PRO
HEA Gra03	0.562**								
Gra04	0.500**								
Gud03	0.262**								
Gud04	0.678**								
Böh03	0.480**								
Böh04	0.021	HEA							
HCM Gra03	0.589**	0.541**							
Gra04	0.663**	0.623**							
Gud03	0.411**	0.384**							
Gud04	0.836**	0.715**							
Böh03	0.586**	0.629**							
Böh04	0.104	0.731**	HCM						
PAN Gra03	-0.248*	-0.416**	-0.288**						
Gra04	-0.258*	-0.371**	-0.346**						
Gud03	-0.047	-0.284**	-0.145						
Gud04	-0.484**	-0.499**	-0.504**						
Böh03	-	-	-						
Böh04	-0.219*	-0.05	-0.066	PAN					
PMI Gra03	-0.463**	-0.463**	-0.569**	0.328**					
Gra04	-0.481**	-0.195	-0.393**	0.249*					
Gud03	-	-	-	-					
Gud04	-0.721**	-0.674**	-0.758**	0.496**					
Böh03	-0.188	-0.465**	-0.438**	-					
Böh04	-0.04	0.067	-0.147	-0.094	PMI				
HUC Gra03	0.477**	0.489**	0.495**	-0.151	-0.314**				
Gra04	0.121	0.154	-0.005	-0.168	0.148				
Gud03	0.243*	0.600**	0.185	-0.259*	-				
Gud04	0.441**	0.591**	0.457**	-0.410**	-0.391**				
Böh03	0.081	0.092	0.044	-	-0.17				
Böh04	-0.077	0.534**	0.238*	-0.061	0.225*	HUC			
TKW Gra03	-0.253*	-0.376**	-0.341**	0.178	0.224*	-0.334**			
Gra04	-0.413**	-0.518**	-0.604**	0.234*	0.158	-0.001			
Gud03	-0.06	-0.424**	-0.14	0.196	-	-0.590**			
Gud04	-0.657**	-0.748**	-0.677**	0.403**	0.606**	-0.620**			
Böh03	0.102	-0.239*	0.076	-	0.156	-0.229*			
Böh04	0.08	-0.706**	-0.463**	0.022	-0.147	-0.793**	TKW		
HLW Gra03	-0.493**	-0.537**	-0.537**	0.279**	0.396**	-0.583**	0.297**		
Gra04	-0.402**	-0.546**	-0.493**	0.208*	0.005	-0.325**	0.394**		
Gud03	-0.232*	-0.694**	-0.296**	0.245*	-	-0.652**	0.601**		
Gud04	-0.470**	-0.532**	-0.484**	0.365**	0.403**	-0.723**	0.731**		
Böh03	-0.149	-0.257*	-0.253*	-	0.529**	-0.352**	0.229*		
Böh04	0.129	-0.616**	-0.304**	0.064	-0.295**	-0.829**	0.874**	HLW	
PRO Gra03	-0.481**	-0.530**	-0.575**	0.256*	0.497**	-0.530**	0.415**	0.456**	
Gra04	-0.545**	-0.462**	-0.589**	0.357**	0.431**	-0.083	0.499**	0.347**	
Gud03	-0.19	-0.485**	-0.239*	0.135	-	-0.487**	0.448**	0.355**	
Gud04	-0.625**	-0.497**	-0.491**	0.366**	0.424**	-0.374**	0.459**	0.303**	
Böh03	-0.703**	-0.585**	-0.701**	-	0.312**	-0.112	0.056	0.157	
Böh04	-0.407**	-0.089	-0.303**	0.182	0.240*	0.236*	-0.244*	-0.401**	PRO
LIP Gra03	0.296**	0.192	0.179	-0.17	-0.075	0.303**	-0.144	-0.148	-0.02
Gra04	0.283**	0.406**	0.411**	-0.217*	-0.209*	-0.014	-0.427**	-0.226*	-0.284**
Gud03	0.310**	0.605**	0.381**	-0.103	-	0.410**	-0.361**	-0.514**	-0.249*
Gud04	0.186	0.338**	0.228*	-0.19	-0.281**	0.178	-0.168	-0.116	0.313**
Böh03	0.365**	0.495**	0.534**	-	-0.447**	0.189	-0.343**	-0.466**	-0.384**
Böh04	0.253*	0.339**	0.356**	-0.079	-0.1	0.222*	-0.273**	-0.11	-0.219*

*, ** Significant at P =0.05 and P =0.01, respectively

Table 5.6 Pearson's correlation coefficients between β-glucan content and other characteristics in different environments

Envir.	Method	YLD	HEA	HCM	PAN	PMI	HUC	TKW	HLW	PRO	LIP
Gra03	NIRS	-0.505**	-0.458**	-0.574**	0.310**	0.540**	-0.304**	0.136	0.466**	0.333**	0.079
	Enzy.	-0.370**	-0.428**	-0.455**	0.172	0.388**	-0.253*	0.103	0.322**	0.224*	-0.231*
Gra04	Enzy.	-0.484**	-0.555**	-0.534**	0.296**	0.303**	-0.226*	0.323**	0.325**	0.434**	-0.440**
Gud03	NIRS	-0.302**	-0.667**	-0.360**	0.196	-	-0.572**	0.496**	0.618**	0.412**	-0.470**
Böh03	NIRS	-0.376**	-0.420**	-0.425**	-	0.324**	-0.184	-0.126	0.2	0.397**	-0.169

*, ** Significant at P =0.05 and P =0.01, respectively

Table 5.7 Relative values to the mean over all entries and environments of standard cultivars, the parents of the AB-QTL-population and the best 20 $BC_2F_{2:6-7}$ lines. Lines are sorted according to the index

Cultivar /line	Relative values to the mean over all environments										Index[a]
	YLD	TKW	PRO	BG(N)	BG(E)	HEA	HCM	PMI	HUC	LIP	
IAH	62	91	118	130	135	96	98	94	106	113	306
'Iltis'	108	101	104	100	89	102	98	102	100	105	379
'Aragon'	121	109	89	92	97	101	93	87	101	91	453
'Freddy'	119	102	86	91	88	103	102	94	108	102	372
'Fl.profi'	118	120	77	88	81	103	102	85	92	89	414
'Marion'	92	112	94	103	114	101	117	68	100	114	344
'Revisor'	113	95	88	95	91	103	100	74	102	92	384
NO1304	121	114	85	92	97	101	100	54	96	79	484
NO1306	122	123	87	93	89	102	98	59	104	88	481
49	100	102	103	103	105	99	90	109	97	95	397
107	94	101	108	102	110	99	88	124	92	95	383
61	97	97	104	103	102	99	90	109	95	93	380
67	98	103	102	106	101	99	89	126	98	97	377
64	97	96	104	105	102	99	94	102	99	97	369
18	106	98	96	99	97	100	102	98	99	101	368
75	97	100	104	104	96	99	87	126	96	95	368
87	102	93	101	98	103	100	100	87	98	100	365
34	90	105	106	103	104	99	89	122	95	91	364
70	94	99	106	107	100	99	89	126	95	94	364
95	110	97	95	92	93	102	103	83	103	99	363
62	107	92	97	102	89	101	101	85	104	102	363
104	95	94	100	102	110	100	100	85	97	94	362
33	101	98	100	100	97	100	105	89	94	102	362
30	99	98	102	102	99	99	91	118	100	99	362
11	90	102	112	103	101	99	90	115	97	94	359
24	96	99	99	103	96	99	91	109	96	102	355
15	107	96	97	94	96	101	102	85	104	102	355
100	108	94	93	97	98	102	103	94	102	101	355
88	104	94	96	102	93	100	101	89	103	99	355

[a] Index= Σ (A) - Σ (B): A= (relative value*weighting factor) of YLD, TKW, PRO, BG(N), BG(E);
B= (relative value*weighting factor) of HEA, HCM, PMI, HUC, LIP

5.1.4 Evaluation of agronomic values

To evaluate the agronomic value of the population 2503, the best 20 $BC_2F_{2\text{-}6:7}$ lines according to the index were compared with novel cultivars and the parents of this population (Table 5.7). There are 3 lines which have higher index value than the parent 'Iltis', but no lines were found with higher values than the novel cultivar 'Aragon' or the breeding lines NO1304 and NO1306. Nevertheless, these best lines showed valuable trait combinations such as high *β*-glucan content, high protein content, low plot height and earlier heading.

5.1.5 Marker polymorphism and segregation

Among 144 oat SSR primer pairs, 24 (16.7%) gave polymorphic bands between the two parents 'Iltis' and IAH611-447. However, only 5 (20.8%) of the 24 polymorphic SSR primer pairs gave segregant bands in the first sub-population. The 5 SSR markers resulting 4 codominant loci and 1 dominant locus, respectively. None of the 5 SSR loci deviated significantly from the expected 1:2:1 or 3:1 segregation ratio at p=0.05 as detected by chi-sq analysis.

For the 256 AFLP primer combinations, 450 polymorphic bands were found between the two parents 'Iltis' and IAH611-447, with an average of about 2 polymorphic bands per primer combination. 105 (23.3%) of 450 markers segregated in the first sub-population and were scored for mapping analysis. Among the 105 AFLP markers scored, 14 were codominant markers, 49 were dominant markers with dominant alleles from the parent IAH611-447, and 42 were dominant markers with dominant alleles from the parent 'Iltis'. Only 3 dominant AFLP markers deviated significantly from the expected 3:1 segregation ratio at p=0.05 as detected by chi-square analysis. None of the 14 codominant AFLP markers deviated significantly from the expected 1:2:1 segregation ratio at p=0.05.

5.1.6 Map development

110 loci were characterized on the 97 lines of the first sub-population 2503. 18 (16.4%) of loci were codominant, including 13 AFLP and 4 SSR loci, respectively. Linkage analysis resulted in 12 linkage groups with 106 loci, whereas 4 loci remained unlinked. The linkage groups contained from 4 to 36 loci and varied in size from 2.7 to 116.0 cM, for a total map size of 455.4 cM (Figure 5.2). The average interval length was 4.6 cM.

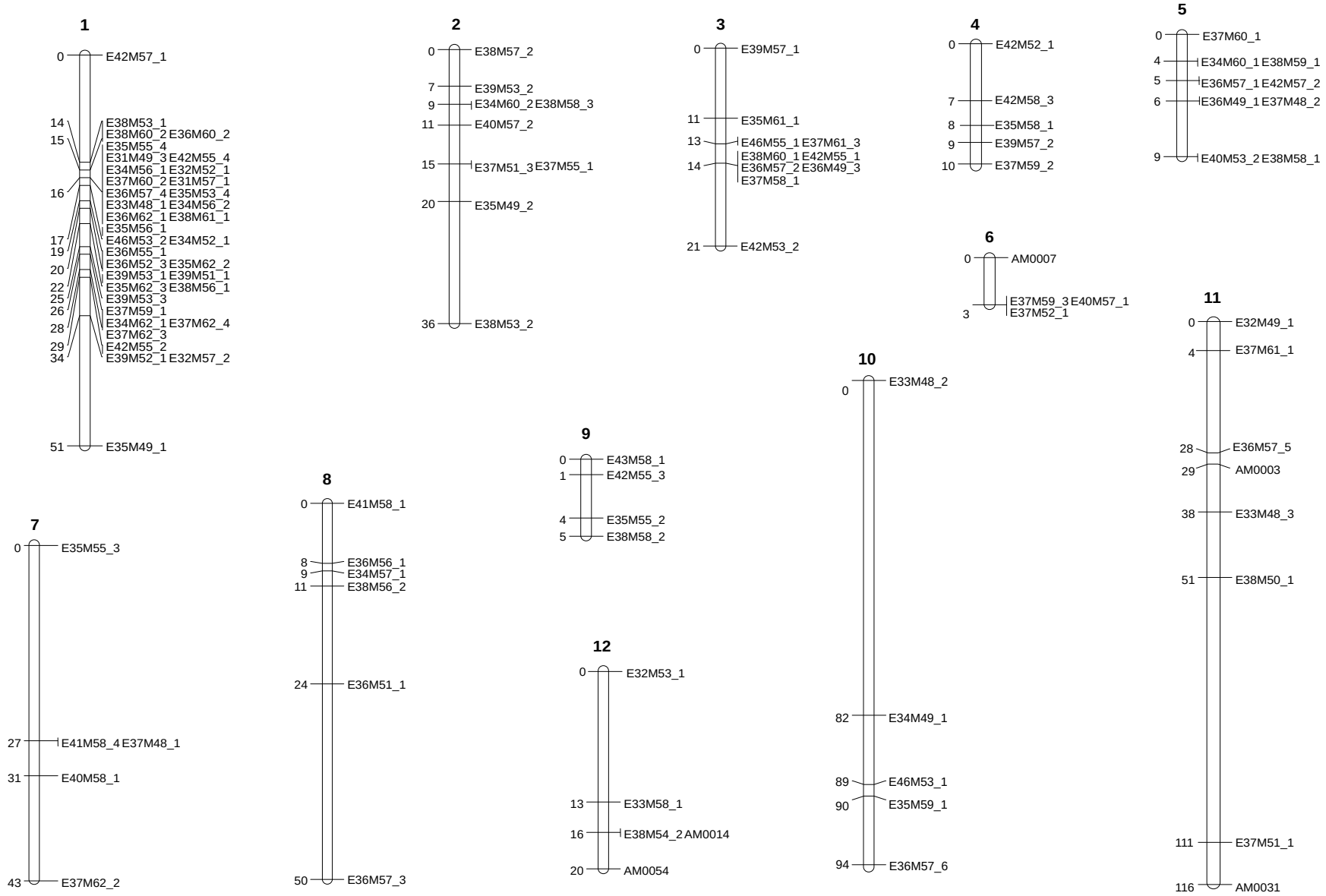

Figure 5.2 Oat molecular linkage map of the sub-population 2503 derived from 'Iltis' x IAH611-447

5.1.7 QTL analysis

Significant QTLs for each trait detected by Simple Interval Mapping (SIM) are listed (Table 5.8). A total of 60 significant QTLs were detected for all 11 traits, ranging from 2 to 9 QTLs/trait. Most QTLs were detected in different environments and are all listed in Table 5.7. For those QTLs detected only in one environment, only a QTL with LOD higher than 3.0 was accepted as significant QTL and listed. QTL with an overlapping support interval are assumed to be the same QTL.

Yield (YLD). Six QTLs were significantly associated with yield on two linkage groups. Five QTLs were detected on linkage group 1 and four of them were detected in two or three environments. For all these five QTLs, the 'Iltis' alleles increased yield and explained from 13.6% to 57.5% of phenotypic variation. Another QTL was detected on linkage group 11 only in one environment (Böh03) with a LOD of 3.29 and the IAH611-447 alleles explained 14.5% of yield increasing.

Height of the plot (HCM). Seven QTLs were detected for height of the plot on 4 different linkage groups. Four QTLs were located on linkage group 1 and the other three QTLs were located on group 2, 4, 11 respectively. For QTLs on group 1 and 4, the 'Iltis' alleles gave positive effect while on group 2 and 11, the IAH611-447 alleles gave positive effect.

Panicle number (PAN). Only two QTLs were detected for panicle number, both on linkage group 1, and the IAH611-447 alleles gave positive effect. QTL Pan1 explained 26.6% of phenotypic variation and was detected only in Gud04 with a LOD of 6.26. QTL pan2 was detected in three different environments and explained phenotypic variation from 16.5% to 27.1%.

Thousand-grain weight (TKW). Eight QTLs were significantly associated with thousand-grain weight on 5 different linkage groups. Five of them were detected in two to six environments, while the other three QTLs were detected only in one environment. Six QTLs (four on group 1, one on group 3 and one on group 4) had positive effects from the IAH611-447 alleles for thousand-grain weight, while the other two QTLs, located on group 5 and 11 respectively, had positive effects from the 'Iltis' alleles.

Test weight (HLW). A total of five significant QTLs were detected for test weight. Four QTLs were located on linkage group 1, and the other one on linkage group 8. For all of these QTLs, the IAH611-447 alleles increased test weight, explaining the phenotypic variation from 13.4% to 53.6%. All of the five QTLs were detected in two to six environments.

Heading (HEA). Three QTLs were detected for heading. Two of them were located on linkage group 1 and were detected in all six environments. Another QTL, located on linkage group 4, was detected in three environments. For all the three QTLs, the 'Iltis' alleles contributed the later heading date. The phenotypic variation explained by the three QTLs ranged from 11.3% to 79.5%.

Hull content (HUC). The highest number of QTLs was found for hull content, totally nine QTLs located on 4 different linkage groups (four on group 1, two on group 3, one on group 8 and two on group 12, respectively). Six QTLs had positive effects from the 'Iltis' alleles on hull content, while the remained three QTLs had positive effects from the IAH611-447 alleles.

Powdery-mildew infection (PMI). Five QTLs were detected for PMI on two linkage groups. Three of them were located on group 1, with enhancing infection effect from the IAH611-447 alleles. The other QTL was located on group 2, with enhancing infection effect from the 'Iltis' alleles, detected only in one location (Gra04) with a LOD of 3.09.

Protein content (PRO). Five QTLs were found significantly associated with protein content on two linkage groups, 1 and 4 respectively. For all of the five QTLs, the IAH611-447 alleles increased the protein content, explaining the phenotypic variation from 13.7% to 57.6%. Three QTLs were detected only in one environment, with a LOD of 7.28 (Pro1), 5.91 (Pro2) and 3.66 (Pro3), respectively.

Lipid content (LIP). Six QTLs were found to be associated with lipid content. All of the six QTLs were located on linkage group 1, with positive effects from the 'Iltis' alles. The phenotypic variation explained by six QTLs ranged from 11.9% to 47.2%.

***β*-glucan content (BG).** For *β*-glucan content, the two measurement methods combined with different locations and years were treated as separate environments. A total of four significant QTLs were detected for *β*-glucan content. All of the four QTLs were located on linkage group 1, and all QTLs had positive effects from the alleles of the donor parent IAH611-447. QTL Bg1 was located on the position of 12-13 cM from the top of linkage group 1. The QTL Bg1 was detected in Gra03 by both NIRS and enzymatic methods, with a LOD of 6.81 and 4.66 respectively. The phenotypic variation explained by QTL Bg1 was 28.6% for NIRS method and 20.6% for enzymatic method. The QTL Bg2 was located around the position of 20-25 cM from the top of linkage group 1, with support intervals from 16 cM to 29cM. The phenotypic variation explained by the QTL ranged from 20.6% to 46%. The QTL Bg3 was located on the position of 34-35 cM. This QTL was detected in three environments, explaining the phenotypic variation from 19.7% to 28.4%. QTL Bg4 was detected on the position of 51 cM of linkage group 1 in two environments with a LOD of 2.52 in Gra04(E), and 3.5 in Gud03(N). The phenotypic variation explained by Bg4 in the two environments was 11.9% and 16.2%, respectively.

Table 5.8 List of significant QTLs detected from data collected from $BC_2F_{2:6-7}$ field plots in 6 environments and average across all environments. The QTL analysis was conducted using simple interval mapping with a LOD threshold of 2.5. Position of QTLs is from the top of the linkage group. Env. = Environment. Supp. IV = support interval. Exp.(%) = explained phenotypic variance obtained from the QTL. Positive values of effect indicate that the recurrent parent 'Iltis' carries favorable alleles; negative values of effect indicate that the donor parent IAH611-447 carries the favourable alleles

Trait	QTL	Env.	Group	Position	Supp.IV	LOD	Exp.(%)	Effect
YLD	Yld1	Böh03	1	16	15-17	6.94	28.1	3.97
	Yld2	Gud03	1	20	17-29	6.07	25	1.61
		Gra03	1	21	18-25	9.89	37.5	4.30
	Yld3	Gra04	1	28	20-29	9.03	34.9	5.48
		Gud04	1	28	24-29	18.02	57.5	6.73
		Average	1	28	24-29	12.52	44.8	3.66
	Yld4	Gra03	1	34	33-35	6.26	25.7	5.12
		Gud03	1	35	34-39	4.01	18.4	1.77
	Yld5	Gra03	1	51	48-51	2.89	13.6	0.82
		Gra04	1	51	48-51	6.01	26.2	2.29
		Gud04	1	51	49-51	6.94	29.6	1.56
		Average	1	51	49-51	5.62	24.8	0.81
	Yld6	Böh03	11	32	27-37	3.29	14.5	-0.59
HCM	Hcm1	Gud03	1	18	16-19	8.38	32.8	6.76
	Hcm2	Gra03	1	25	24-29	22.01	64.8	6.51
		Böh03	1	25	24-26	16.92	55.2	7.76
		Gud04	1	26	24-29	25.09	69.6	7.72
		Gra04	1	28	24-29	26.9	72.1	8.88
		Böh04	1	28	24-29	19.83	61	7.89
		Average	1	28	24-29	31.05	77.1	6.89
	Hcm3	Gud03	1	35	34-39	5.08	22.7	2.34
	Hcm4	Gra03	1	51	49-51	8.41	34.7	2.50
		Gra04	1	51	49-51	8.28	34.2	3.52
		Gud04	1	51	49-51	11.38	43.8	3.86
		Böh03	1	51	49-51	6.61	28.5	1.35
		Böh04	1	51	49-51	8.72	35.7	4.59
		Average	1	51	49-51	11.06	42.9	2.83
	Hcm5	Böh04	2	31	15-35	2.81	12.5	-4.50
		Gud03	2	35	25-35	4.08	17.6	-2.79
		Average	2	32	15-35	2.52	11.3	-3.56
	Hcm6	Gud04	4	9	7-9	2.65	11.8	0.97
		Böh04	4	9	8-9	2.87	12.8	0.58
		Average	4	9	7-9	2.83	12.6	0.80
	Hcm7	Böh03	11	28	27-32	3.74	16.3	-2.93
PAN	Pan1	Gud04	1	9	3-15	6.26	26.6	-39.96
	Pan2	Gra03	1	25	22-29	3.79	16.5	-25.71
		Gra04	1	25	22-29	3.87	16.8	-24.49
		Gud04	1	25	24-29	6.65	27.1	-40.69
		Average	1	25	24-29	11.03	40.8	-25.60

Table 5.8 (continued)

Trait	QTL	Env.	Group	Position	Supp. IV	LOD	Exp. (%)	Effect
TKW	Tkw1	Böh04	1	9	4-15	11.84	44.4	-2.64
	Tkw2	Böh04	1	21	20-26	13.56	47.5	-2.56
		Gud04	1	22	20-29	13.92	48.4	-2.27
		Böh03	1	23	20-26	3.05	13.5	-0.71
		Gra03	1	25	22-29	4.5	19.2	-0.98
		Gra04	1	25	20-29	12.02	43.5	-1.26
		Gud03	1	25	20-29	4.4	18.9	-1.24
		Average	1	25	20-26	16.83	55	-1.48
	Tkw3	Gud04	1	35	34-37	11.56	44.3	-2.34
		Böh04	1	35	34-38	6.8	29.1	-2.17
	Tkw4	Gra04	1	51	49-51	6.2	26.9	-0.28
		Gud04	1	51	49-51	8.64	35.4	-0.55
		Böh04	1	51	49-51	5.14	22.9	-0.73
		Average	1	51	49-51	7.71	32.3	-0.47
	Tkw5	Böh03	3	16	13-19	3.18	14	-1.11
	Tkw6	Gud04	4	9	8-9	2.72	12.1	-0.07
		Böh04	4	9	7-9	3	13.3	-0.06
	Tkw7	Gud04	5	5	4-7	3.53	15.5	1.10
		Böh03	5	5	4-6	3.51	15.3	0.60
	Tkw8	Böh03	11	28	27-33	3.97	17.2	0.09
HLW	Hlw1	Gra04	1	6	0-13	6.84	28.7	-0.81
		Böh03	1	6	0-16	2.91	13.4	-0.46
		Gra03	1	7	0-13	7.45	30.8	-0.76
		Böh04	1	9	2-16	7.15	29.8	-1.92
		Average	1	8	4-13	11.96	44.7	-1.00
	Hlw2	Gud04	1	20	17-29	5.92	24.5	-0.95
		Böh04	1	21	20-25	9.3	35.7	-1.96
		Gra04	1	23	20-29	6.62	27	-0.70
		Böh03	1	24	20-25	4.58	19.5	-0.68
		Gra03	1	25	20-29	10.31	38.7	-0.76
		Gud03	1	25	24-29	12.37	44.4	-1.10
		Average	1	21	20-25	16.19	53.6	-1.03
	Hlw3	Gud04	1	35	34-38	5.41	24	-1.08
		Böh03	1	35	34-40	3.76	17.3	-0.66
		Böh04	1	35	33-40	4.23	19.3	-1.56
		Average	1	35	34-37	9.26	37.4	-0.92
	Hlw4	Gra03	1	51	48-51	5.75	25.2	-0.36
		Gud03	1	51	48-51	4.33	19.7	-0.34
		Gud04	1	51	48-51	3.92	18	-0.14
		Böh04	1	51	47-51	2.9	13.7	-0.51
		Average	1	51	49-51	5.98	26.1	-0.28
	Hlw5	Gud04	8	5	0-18	2.68	11.9	-2.77
		Gud03	8	7	1-8	3.18	14	-2.70
		Average	8	6	0-12	2.66	11.9	-1.88
HEA	Hea1	Gra04	1	25	24-26	24.44	68.7	2.83
		Gud03	1	25	24-26	31.7	77.8	2.50
		Gud04	1	25	24-26	28.17	73.8	3.20
		Böh03	1	25	24-26	25.53	70.3	2.22
		Böh04	1	25	24-26	30.45	76.4	3.23
		Gra03	1	26	25-29	27.56	73	2.50
		Average	1	25	24-26	33.42	79.5	2.74

Table 5.8 (continued)

Trait	QTL	Env.	Group	Position	Supp.IV	LOD	Exp.(%)	Effect
HEA	Hea2	Gra03	1	51	49-51	5.49	24.3	0.75
		Gra04	1	51	50-51	7.19	30.5	1.00
		Gud03	1	51	50-51	10.09	40	0.69
		Gud04	1	51	50-51	9.55	38.3	0.88
		Böh03	1	51	49-51	4.95	22.1	0.45
		Böh04	1	51	49-51	10.39	40.9	1.05
		Average	1	51	50-51	9.23	37.3	0.79
	Hea3	Gud03	4	9	8-9	2.84	12.6	0.05
		Böh03	4	9	7-9	2.53	11.3	0.16
		Böh04	4	9	7-9	3.01	13.3	0.45
		Average	4	9	7-9	2.79	12.4	0.20
HUC	Huc1	Gra03	1	20	18-26	7.8	31	0.75
		Gud04	1	20	16-21	7.75	30.8	1.02
		Böh04	1	21	20-25	7.85	31.1	2.04
		Average	1	22	18-25	11.77	42.8	0.95
	Huc2	Gud03	1	28	24-29	8.43	33	1.69
		Böh03	1	29	28-30	3.26	14.3	13.64
	Huc3	Gra03	1	34	33-35	6.28	25.8	0.93
		Gud04	1	34	33-35	4.97	21	1.29
		Böh04	1	34	33-36	3.39	14.9	1.98
		Average	1	34	33-35	7.74	30.8	1.15
	Huc4	Gra03	1	51	48-51	3.09	14.5	0.25
		Gud03	1	51	48-51	3.56	16.5	0.42
		Average	1	51	48-51	3.54	16.4	0.27
	Huc5	Gra04	3	0	0-4	3.08	13.6	-1.11
	Huc6	Gra03	3	16	13-19	2.69	12	2.27
		Böh03	3	19	16-21	4.83	20.5	2.62
		Average	3	16	13-19	2.53	11.3	2.50
	Huc7	Gud04	8	5	0-8	4.1	17.7	3.69
		Böh03	8	5	0-9	3.6	15.7	1.03
		Average	8	7	0-8	2.72	12.1	1.92
	Huc8	Gra03	12	0	0-11	2.83	12.6	-0.54
		Gra04	12	0	0-9	2.84	12.6	-0.39
	Huc9	Gra03	12	19	16-19	3.41	18.4	-0.52
PMI	Pmi1	Böh03	1	4	0-10	7.01	29.3	-0.53
	Pmi2	Gra04	1	23	20-29	4.47	19.1	-0.37
		Gud04	1	25	24-29	13.91	48.3	-0.94
		Gra03	1	26	24-29	11.05	40.8	-1.01
		Böh03	1	28	24-29	10.68	39.8	-0.62
		Average	1	28	24-29	17.09	55.6	-0.61
	Pmi3	Gra04	1	35	34-41	5.87	25.7	-0.51
	Pmi4	Gra03	1	51	46-51	3.76	17.3	-0.46
		Gra04	1	51	44-51	5.25	23.3	-0.34
		Gud04	1	51	47-51	3.79	17.5	-0.50
		Böh03	1	51	48-51	4.36	19.8	-0.24
		Average	1	51	49-51	7.48	31.5	-0.34
	Pmi5	Gra04	2	35	25-35	3.09	13.7	0.51

Table 5.8 (continued)

Trait	QTL	Env.	Group	Position	Supp. IV	LOD	Exp. (%)	Effect
PRO	Pro1	Gud04	1	7	1-13	7.28	30.3	-1.10
	Pro2	Böh04	1	18	16-21	3.09	13.7	-1.89
		Gra04	1	19	18-21	13.03	46.1	-1.08
	Pro3	Gra03	1	25	24-29	16.05	53.3	-0.84
		Böh03	1	25	22-26	8.62	33.6	-0.96
		Gud03	1	26	20-29	6.82	27.7	-0.62
		Gud04	1	28	20-29	7.68	30.6	-1.08
		Average	1	25	24-29	18.09	57.6	-0.81
	Pro4	Gra04	1	35	34-37	5.91	25.8	-0.94
	Pro5	Böh03	4	9	7-9	3.66	16	-0.20
LIP	Lip1	Gra04	1	5	0-13	4.84	21.3	0.23
		Böh04	1	6	0-13	6.46	27.4	0.21
	Lip2	Gud03	1	16	14-17	10.42	39	0.29
		Average	1	15	14-17	10.79	40.1	0.22
	Lip3	Böh03	1	23	22-25	13.46	47.2	0.33
	Lip4	Gra04	1	28	24-29	5.78	24	0.25
		Gud03	1	28	18-29	9.51	36.3	0.32
		Average	1	28	18-29	10.57	39.5	0.24
	Lip5	Böh03	1	35	34-38	6.68	28.7	0.28
		Gud04	1	41	33-45	3.88	17.8	0.43
	Lip6	Gra04	1	51	45-51	2.51	11.9	0.10
		Gud03	1	51	49-51	4.43	20.1	0.04
		Böh03	1	51	49-51	6.84	29.3	0.06
		Average	1	51	48-51	3.82	17.6	0.08
BG	Bg1	Gra03(E)	1	12	4-17	4.66	20.6	-0.23
		Gra03(N)	1	13	6-16	6.81	28.6	-0.17
	Bg2	Böh03(N)	1	20	18-21	6.91	28	-0.14
		Gra04(E)	1	21	20-25	8.89	34.4	-0.16
		Gud03(N)	1	22	20-25	12.28	44.2	-0.25
		Gra03(E)	1	24	17-26	4.87	20.6	-0.24
		Gra03(N)	1	25	16-29	6.63	27	-0.16
		Average(E)	1	24	18-25	8.89	34.4	-0.19
		Average(N)	1	22	20-25	12.99	46	-0.17
	Bg3	Gra04(E)	1	34	33-35	5.32	22.3	-0.16
		Gud03(N)	1	34	33-38	7.05	28.4	-0.28
		Böh03(N)	1	35	34-42	4.34	19.7	-0.13
		Average(N)	1	35	34-39	7.42	31.3	-0.16
		Average(E)	1	35	33-42	3.2	14.9	-0.13
	Bg4	Gra04(E)	1	51	48-51	2.51	11.9	-0.02
		Gud03(N)	1	51	48-51	3.5	16.2	-0.07
		Average(N)	1	51	48-51	4.08	18.7	-0.06

5.2 Genetic analysis of oat powdery-mildew resistance

5.2.1 Inheritance of the powdery-mildew resistance

The two BC_2F_7 lines, Am27 and Am28, used as parents for the third backcross, showed a nearly complete resistance (score 1) (Table 5.9) to all powdery-mildew isolates in leaf-segment tests and field experiments at different years and locations. The infection of F_1, and the segregation patterns of F_2, F_3 and BC_1 populations are shown in Table 5.9. In the cross of Am27 x 'Neklan', the observed segregation pattern in the F_2 population with 277 resistant and 105 susceptible plants fits a 3:1 ratio, supporting the hypothesis that the powdery-mildew resistance in Am27 is controlled by a single dominant gene. This was confirmed by the segregation patterns of 300 F_3 families with 84 nonsegregating resistant families, 136 segregating families and 80 nonsegregating susceptible families, which fits a 1:2:1 F_2 genotypic ratio (χ^2=2.72). The single-dominant gene hypothesis was further supported by analysis of a BC_1 population, in which a 1R:1S segregation was observed, and by the segregation analysis of F_2 and F_3 populations of Am28 x 'Flämingsprofi'/'Neklan' (Table 5.9). Comparing the nearly completely resistant parents Am27 and Am28 with the F_1, F_2 and F_3, several individuals of the segregating populations showed scores of *2*, indicating an incomplete gene action of this dominant gene. This dominant powdery-mildew resistance gene derived from *A. macrostachya* was tentatively designated *Eg5* according to the nomenclature of oat genes (Simmons et al. 1978) and the summary of resistance sources by Herrmann and Roderick (1996).

The phenotypic grouping of scores 0-2 and 3-5 as resistant and susceptible reaction patterns, respectively, was genotypically validated by testing F_3 offspring. Among a total of 480 F_2 plants tested, all F_2 plants with reactions scored 0-2 either gave nonsegregating resistant or segregating F_3 progeny, while F_2 plants displaying reactions scored 3 or higher invariantly led to susceptible F_3 progeny. Thus, the grouping of scores reflected the underlying resistance genotypes, i.e., *Eg5Eg5* or *Eg5eg5* vs. *eg5eg5*. Figure 5.3 is a sample of detached-leaf tests on benzimidazole-containing agar.

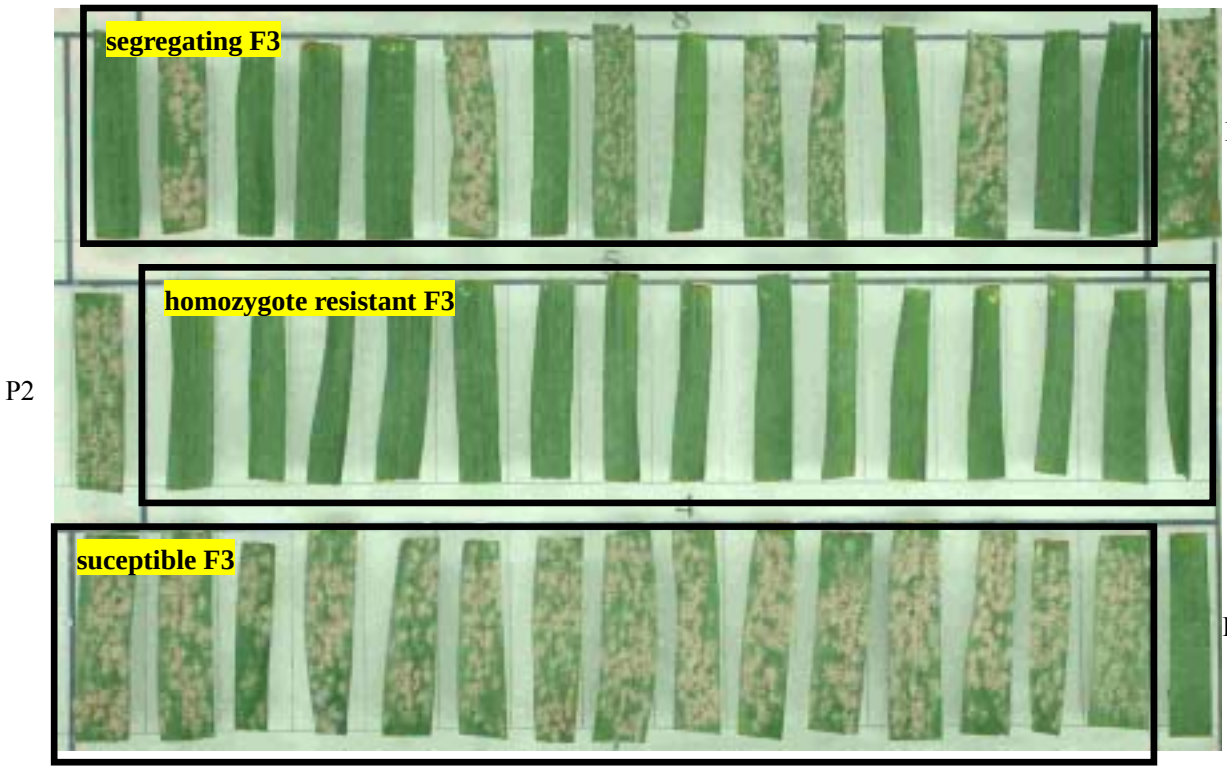

Figure 5.3 Leaf segments of F2-3 families of the cross Am27 x 'Neklan' in the detached leaf segment test 9 days post inoculation (P1: Am27; P2: 'Neklan')

Table 5.9 Reactions of parents, F_1, F_2, F_3 and BC_1 of crosses with powdery-mildew resistant lines Am27 and Am28 in leaf-segment tests

Generation	**Resistant**		**Segregating**	**Susceptible**			**Ratio fit**	**χ^2 value**	**P**
Score	**0+1**	**2**		**3**	**4**	**5**			
'Neklan'					6	5			
'Flämingsprofi'				1	9	1			
Am27/Am28	15								
Am27 x 'Neklan'									
F_1	7	1							
F_2	241	36		21	84		3 : 1	1.26	0.26
BC_1	67	38			97	13	1 : 1	0.12	0.73
F_3		84	136			80	1 : 2 : 1	2.72	0.26
Am28 x 'Neklan'									
F_1	4	14							
F_2	101	125		0	31	28	3 : 1	2.81	0.09
Am28 x 'Flämingsprofi'									
F_1	7	11							
F_2	202	24		1	60	9	3 : 1	0.29	0.59
F_3		49	83			48	1 : 2 : 1	1.1	0.58

5.2.2 SSR polymorphism and BSA analysis

A total of 144 SSR primers were screened to identify polymorphisms between resistant parent Am28 and susceptible parent 'Flämingsprofi'. 16 primers (11%) gave distinguishable polymorphic patterns and were used for further bulk analysis. Only one SSR primer, AM102, generated polymorphic fragments between the two bulks as shown in Figure 5.4. Fragments amplified in resistant parent and susceptible parent using primers AM102F (5' TGGTCAGCAAGCATCACAAT 3') and AM102R (5' TGTGCATGCATCTGTGCTTA 3') were 201 bp and 213 bp in size, respectively. This SSR-primer pair was then used for genotyping of the two F_3 populations. Figure 5.5 presents as a sample the segregation patterns of SSR marker AM102.

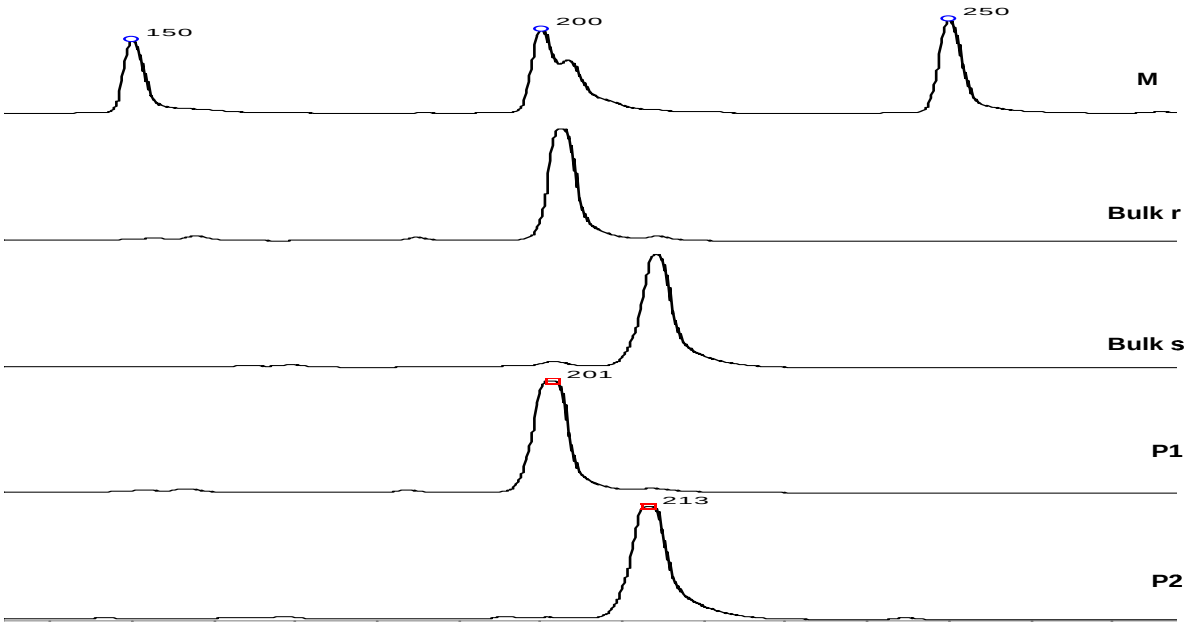

Figure 5.4 Bulked-segregant analysis of SSR marker AM102 using Fragment Analyser version 1.00. M: external size standards, Bulk r: resistant bulk, Bulk s: susceptible bulk, P1: resistant parent Am28, P2: susceptible parent 'Flämingsprofi'. The horizontal scale indicates fragment sizes in base pairs

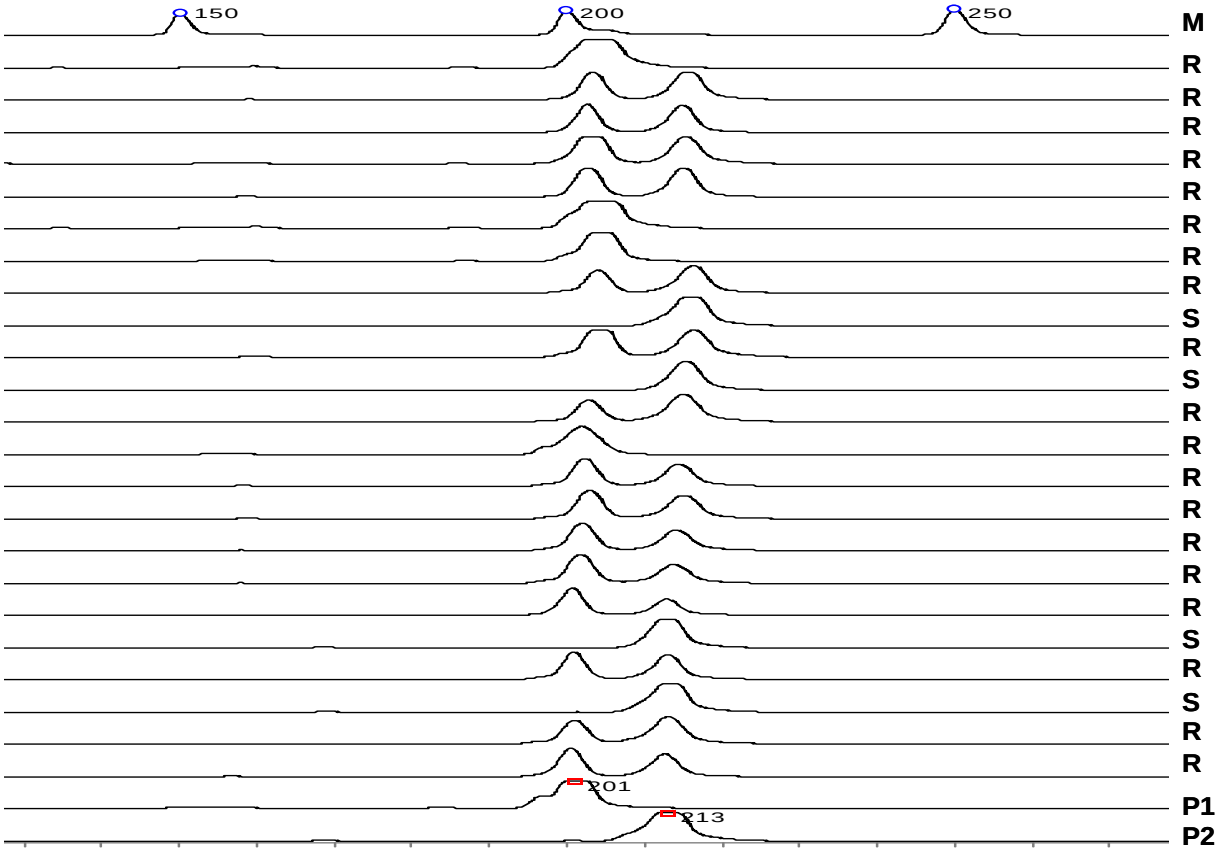

Figure 5.5 Segregation patterns of the SSR marker AM102 in a subset of 23 $F_{2:3}$ families. M: external size standards, R: resistant, S: susceptible, P1: resistant parent Am28, P2: susceptible parent 'Flämingsprofi'. The horizontal scale indicates fragment sizes in base pairs

5.2.3 AFLP polymorphisms and BSA analysis

A total of 256 AFLP selective primer combinations were screened to identify polymorphisms between resistant parent Am28 and susceptible parent 'Flämingsprofi', and between two bulks. 21 AFLP bands were polymorphism between the two parents, and the two bulks. The polymorphisms were further confirmed in the 180 F_3 families. 8 AFLP markers which gave clear strong polymorphic patterns, including one codominant marker, were chosen for STS-marker development. Figure 5.6 displays the polymorphism banding patterns exhibited by E41M56.

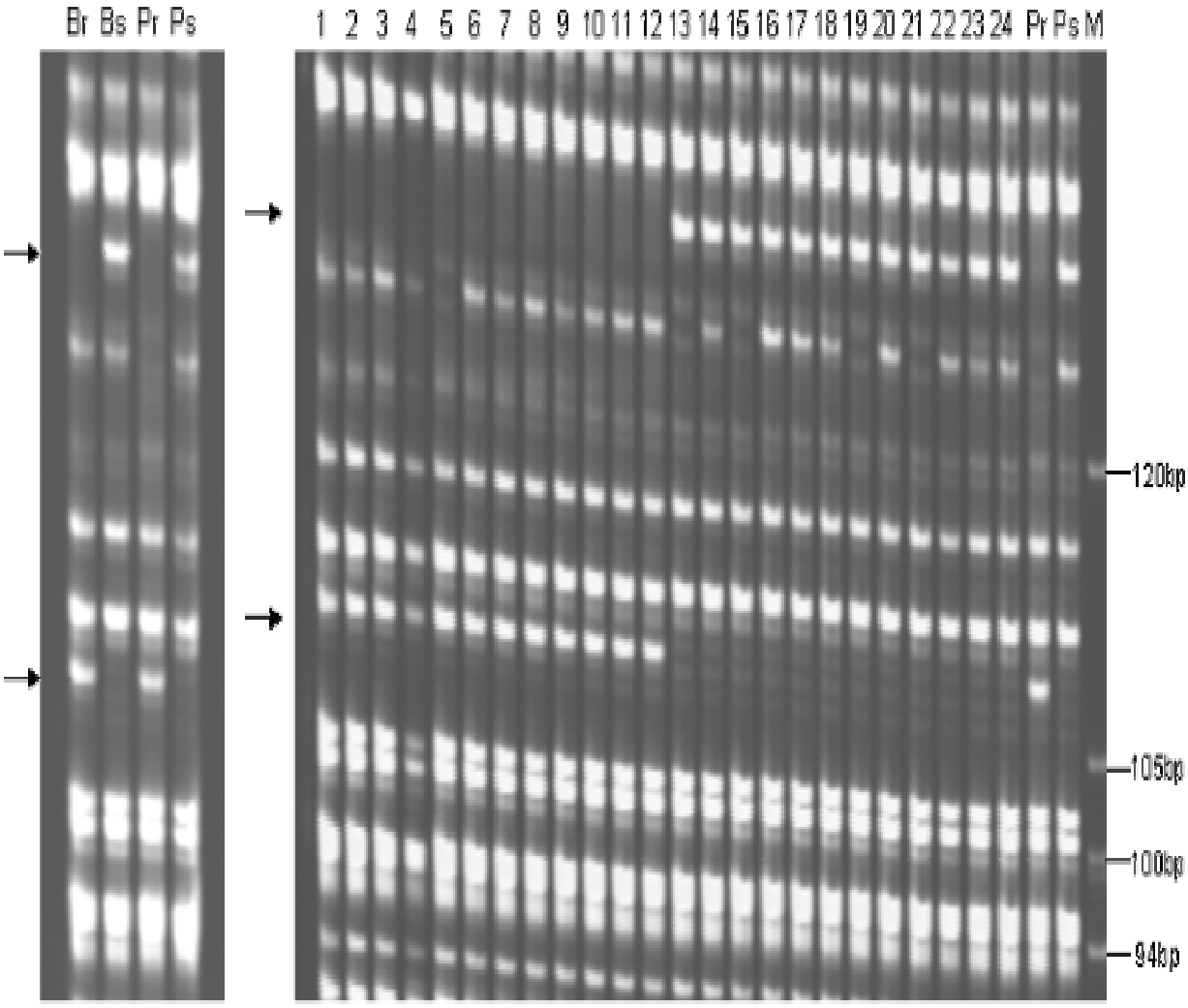

Figure 5.6 Polymorphsims detected between the two bulks and among the F_3 families using AFLP primer combination E41M56. Br: resistant bulk; Bs: susceptible bulk; Pr: resistant parent Am28; Ps: susceptible parent 'Flämingsprofi'. Lines: 1-12 resistant F_3 families; 13-24 susceptible F_3 families; M molecular weight marker. The PCR products were separated on a LI-COR automated sequencer using a KB^{Plus} 6.5% gel matrix

5.2.4 Cloning and conversion of AFLP markers

For the 8 selected AFLP markers, totally 9 bands were recovered and cloned (Figure 5.7). Clones were confirmed by PCR using the corresponding AFLP primer combinations. Figure 5.7 shows the PCR results of 4 cloned AFLP polymorphic markers, including one codominant marker E41M56 which has both resistant and susceptible alleles. For each AFLP band, 2 confirmed clones were sequenced. Sequence comparison of the two alleles of the codominant AFLP marker E41M56 revealed polymorphisms including five SNPs and one indel (21bp) (Figure 5.8). For all dominant AFLP markers, alignments of sequences of the two clones

revealed 100% identity except one marker E37M47 which showed different sequences indicating different bands shared the same size and the same selective bases for this marker. Primer sets were designed for each AFLP markers according the sequence information.

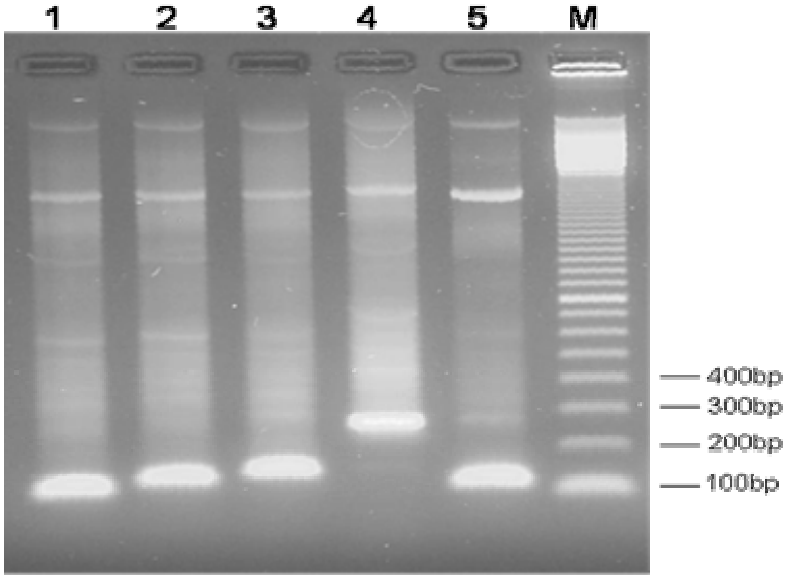

Figure 5.7 PCR results of cloned AFLP polymorphic fragments using corresponding AFLP primer combinations. 1: resistant allele of codominant AFLP marker E41M56; 2: susceptible allele of codominant AFLP marker E41M56: 3: resistant allele of dominant AFLP marker E45M56; 4: resistant allele of dominant AFLP marker E41M61; 5: resistant allele of dominant AFLP marker E36M55; M molecular weight marker. The PCR products were separated on 1.2% agrose gel

```
1  GAATTCAGGCATAAAACTTGTCGAGACGAATTGGAAGATGCATTTTCCGGGAGGGCCGCA
   ||||||||||||||||||||||   |                      ||  |||||||
1  GAATTCAGGCATAAAACTTGTCGATGCC.....................GGGAGGCCGCA

61 GATACGTGTTATCACACGGTAGATCAGATCAGATAAGGACGGCGTTAA
    |||||||||||||||||||| ||||||||||||||||||||||||||
40 GATACGTGTTATCACACGGTATATCAGATCAGATAAGGACGGCGTTAA
```

Figure 5.8 Nucleotide sequences of both resistant and susceptible alleles of the converted codominant AFLP marker E41M56. Underlined nucleotides are the sequences used for designing AFLP-derived STS marker ASE41M56 primers

All primer sets derived from AFLP markers were used to check polymorphism between 5 resistant and 5 susceptible individuals, and the two parents for both populations. Four primer sets (named as ASE41M56, ASE45M56, ASE41M61 and ASE36M55) revealed the same polymorphism patterns as corresponding AFLP markers in the population of Am28 x 'Flämingsprofi' (Figure 5.9) and three primer sets (ASE41M56, ASE45M56 and ASE36M55)

revealed the same polymorphism patterns as corresponding AFLP markers in the population of Am27 x 'Neklan'. These primer sets (Table 5.10) were then used to screen the whole populations for linkage analysis respectively.

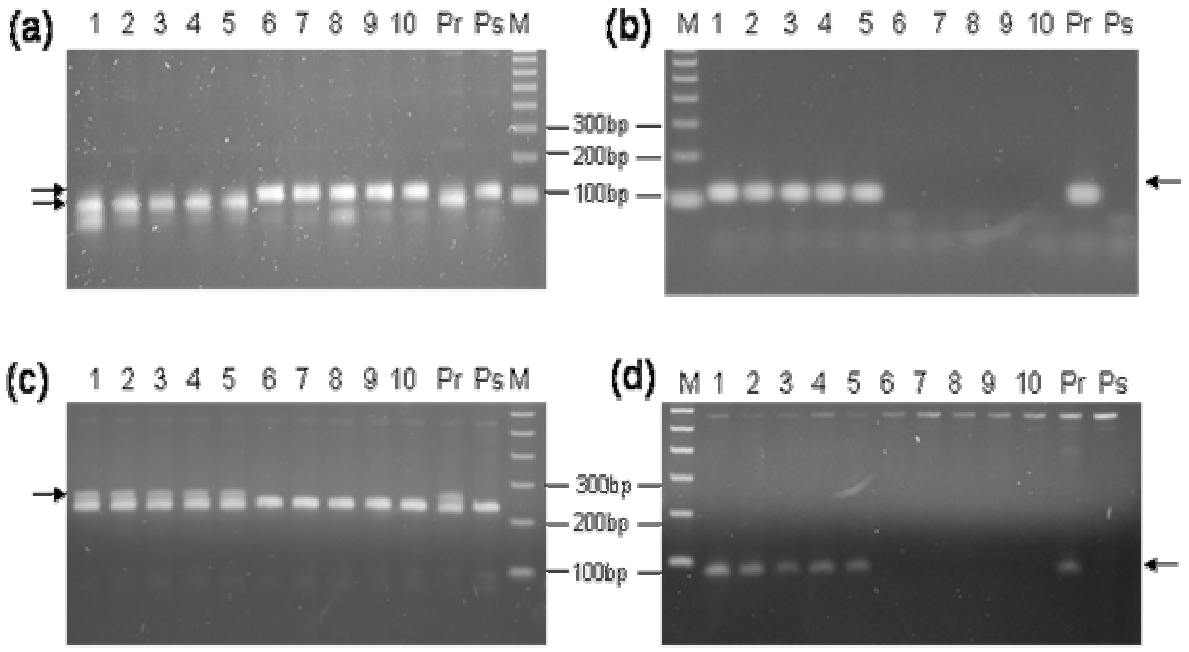

Figure 5.9 The PCR products amplified by the STS primers ASE41M56 (a), ASE45M56 (b), ASE41M61 (c) and ASE36M55 (d) among the two parents and the F_3 families from Am28 x 'Flämingsprofi'. Pr: resistant parent Am28; Ps: susceptible parent 'Flämingsprofi'. Lines: 1-5 resistant F_3 families; 6-10 susceptible F_3 families; M molecular weight marker. The PCR products were separated on 1.2% agrose gel.

Table 5.10 AFLP-derived STS marker primer sequences, amplified fragment length and PCR melting temperatures

STS marker	Primer pair sequence	Product size (bp)	Tm ^{o}C
ASE41M56	5' GAATTCAGGCATAAAACTTGTCG 3'	85	58
	5' AACGCCGTCCTTATCTGATCT 3'		
ASE45M56	5' TTCATGTATAAGTCCTTAGTTTTATCG 3'	122	56
	5' CATAGCAACACGCACGAAGT 3'		
ASE41M61	5' TTCAGGTGGGCTAATCTGGT 3'	245	55
	5' ACCACTCACAACCCTTGCTT 3'		
ASE36M55	5' CCATCAGATTAGTCAAGGTCACA 3'	95	55
	5' CAAAGTATAGCTAAACCGTTGAAA 3'		

5.2.5 Linkage analysis

Am28 x 'Flämingsprofi' population. Among 180 F_3 families screened, only two recombinant families, which were homozygous resistant but heterozygous for SSR marker AM102, were found, indicating that AM102 is tightly linked to the powdery-mildew resistance gene. The genetic distance was then calculated using Joinmap 3.0 program (Van Ooijen and Voorrips, 2001), and a genetic distance of 0.4 cM was obtained. The AFLP-derived codominant STS markers ASE41M56-87 co-segregated with SSR marker AM102. 3 AFLP-derived dominant STS markers (ASE45M56-122, ASE41M61-245, ASE36M55-95) co-segregated with the powdery-mildew resistance gene (Figure 5.10A).

Am27 x 'Neklan' population. The SSR marker AM102 and the AFLP-derived codominant STS markers E41M56-87 revealed the same segregating patterns, with a genetic distance of 2 cM to the powdery-mildew resistance gene. The 3 AFLP-derived dominant STS markers (ASE45M56-122, ASE36M55-95) revealed the same loci linked to the powdery-mildew resistance gene with a genetic distance of 1 cM (Figure 5.10B).

Comparative mapping. The SSR marker AM102 and the 4 AFLP-derived STS markers were used to screen the 71 F_6-derived RI lines of KO population. Only the SSR marker AM102 revealed polymorphisms in the population. Since Pal et al. (2002) assigned AM102 to the linkage group 22_44+18 in KO mapping population, we selected the framework markers of this linkage group to analysis the linkage with SSR marker AM102. The result revealed that SSR marker AM102 mapped between RFLP marker cdo419 and cdo484a in the KO-22_44+18 linkage group, with a genetic distance of 1 cM to cdo419, and 3 cM to cdo484a (Figure 5.10C).

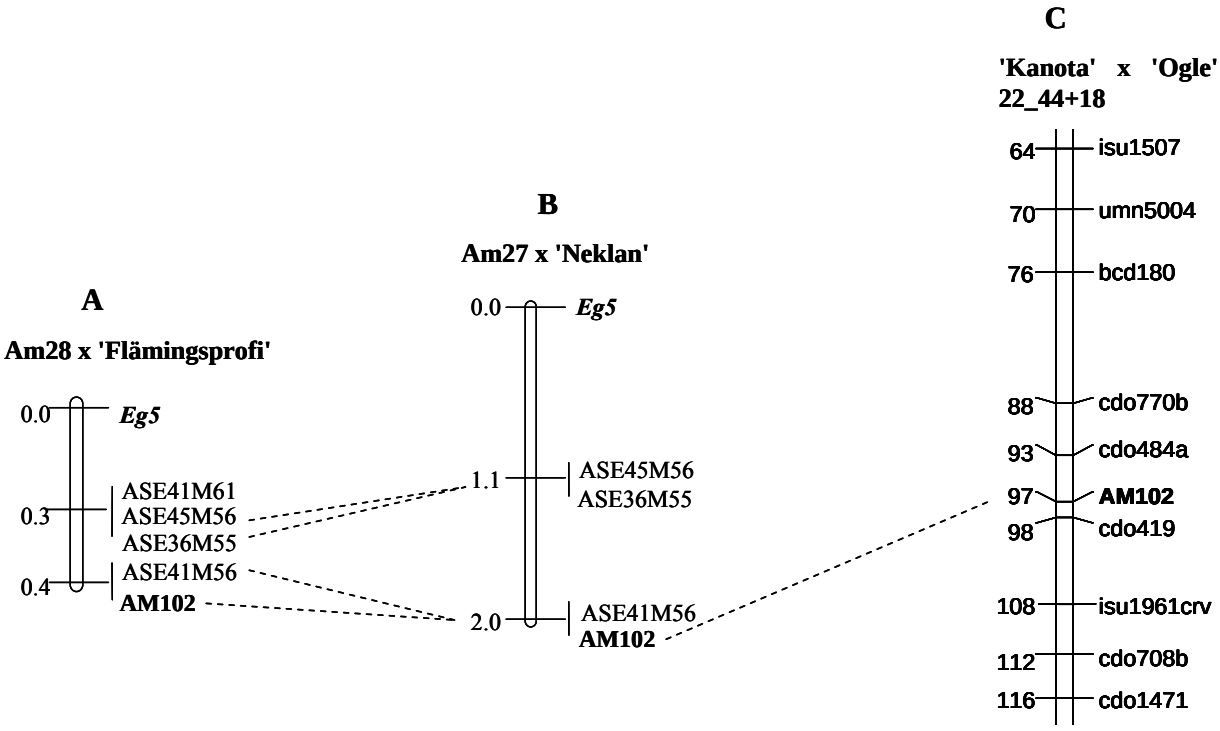

Figure 5.10 A-C Genetic mapping of the dominant resistance gene *Eg5* derived from *Avena macrostachya*. **A** Linkage map around *Eg5* from Am28 x 'Flämingsprofi'; **B** Linkage map around *Eg5* from Am27 x 'Neklan'; **C** Linkage map around SSR marker AM102 from KO-22_44+18 linkage group

6 Discussion

6.1 AB-QTL analysis

6.1.1 Phenotypic distribution

In order to analyse the phenotypic distribution, the correlation coefficients between different environments for each trait were investigated firstly. The results showed a significant positive correlation coefficient between different environments for most traits. Based on this result, it was decided to analyse the distribution of each trait using the mean values across all environments.

As shown in Figure 5.1, nearly all the traits evaluated gave continuous distribution patterns around the mean values, although several traits (yield, height of the plot, panicle number, powdery-mildew infection, protein content and *β*-glucan content) showed significant skewnesses. The continuous distribution patterns confirmed the quantitative character of the traits evaluated. However, the mean values of most traits showed skewnesses or a shift to the recurrent parent 'Iltis'. These results are not surprising since the $BC_2F_{2:6-7}$ population investigated here was derived from a single BC_2 seed, and theoretically, this population is expected to have 87.5% of the recurrent parent genome recovered.

Since evaluation of *β*-glucan contents had been performed in BC_1F_2 plants and subsequently the progenies with the highest *β*-glucan content were selected for the population development, it was expected to have a population with a wide range lines including those lines which have equal values to the high *β*-glucan parent. Unfortunately, a shift of the mean values to the recurrent parent 'Iltis' which has lower *β*-glucan content was found for *β*-glucan content values using both NIRS and enzymatic assay methods of measurement (Figure 5.1 and Table 5.2). This may be due to the same reason that the population was developed from a single BC_2 seed and only limited genomic regions from the higher *β*-glucan donor parent IAH611-447 were successfully introgressed into the BC_2F_2 population. Therefore, part of the alleles which have beneficial influences on *β*-glucan content from IAH611-447 may have been lost. Additionally, it is known that the *β*-glucan content is controlled by several genes (Holthaus et

al., 1996; Kibite and Edney, 1998). Therefore, it is probable that segregation of these genes occurs within the BC_2F_3 generation, leading to more or less heterogeneity in each BC_2F_5 line. Such heterogeneity was visible concerning height and heading, and it may be also the same case for β-glucan content.

6.1.2 Trait correlation

Some of the correlations found in this work are specific for the $BC_2F_{2:6-7}$ population because the two parents showed striking differences in yield, heading, panicle number, thousand-grain weight, protein and β-glucan content. The higher yield of 'Iltis' is realised mainly by higher kernel numbers per panicle and slightly higher thousand kernel weights, but a lower number of panicles per square meter. To appraise the negative correlation between β-glucan content and yield in this study, one has to consider the possible linkage between the high β-glucan content and the other traits of the parent IAH611-447, especially the high number of panicles and the low number of kernels per plant. The latter one has not been measured in this study, but from unpublished experiments IAH611-447 plants yielded only half of the kernel number as 'Iltis' (Herrmann 2005, personal communication). Considering two backcrosses were performed and theoretically only 12.5 % IAH611-447 derived segments were remained in the genome of the population lines, it is not surprising that there are only limited lines which have high β-glucan content and high panicle number, but low kernel numbers per plant.

The possible reasons (pleiotropic effects, linkage of genes) for the negative correlation between yield and β-glucan can’t be evaluated properly with the available data because the linkage map of the population is not comprehensive and dense enough to get reliable values of all QTLs for yield (see chapter 5.1.7), yield components and β-glucan content. According to the relatively low correlation coefficients in current study, together with results from other studies, it seems that the high β-glucan content is not linked with high panicle number or yield, and it should be possible to get lines combined with high yield and higher β-glucan content. The yield may be based on a modified yield structure which has higher panicle numbers and perhaps similar kernel number per panicle as the parent line 'Iltis'. But among the current population there is no line significantly out-yielding the parent 'Iltis'. Most of the high yielding lines have rather low panicle numbers as 'Iltis'. This can be also reflected by a

negative correlation coefficient between yield and panicle number. Additionally, most of the high yielding lines possess taller culms up to 10cm in comparison with 'Iltis', increasing the risks of lodging. Furthermore, the lines of this population need to be compared with new cultivars, which were included in the field trials.

Table 6.1 Coefficients of correlations of β-glucan with other traits.

Trait	Correlation[1]	Plant material investigated	References
Protein	-	Wild oat	Miller et al. (1993a)
Protein	-/o/+	Cultivated oats	Saastamoinen et al. (1992)
Protein	o	Cultivated oats	Peterson (1992)
Protein	o	Cultivated oats	Baur (1995)
Protein	+/o	Cultivated oats	Kibite and Edney (1992)
Protein	+	within oat genotypes	Welch et al. (1991)
Protein	+	Cultivated oats	Miller et al. (1993b)
Protein	+	Cultivated oats	Brunner and Freed (1994)
Protein	+	Cultivated oats	Cervantes-Martinez et al. 2002
Oil content	-	Cultivated oats	Welch and Lloyd (1989)
Oil content	-	Cultivated oats	Kibite and Edney (1992)
Oil content	-	Cultivated oats	Cervantes-Martinez et al. 2002
Hull content	-	Cultivated oats	Saastamoinen et al. (1992)
Hull content	o	Cultivated oats	Brunner and Freed (1994)
Hull content	-	Cultivated oats	Holthaus et al. (1996)
1000-grain weight	+	Cultivated oats	Saastamoinen et al. (1992)
1000-grain weight	+/o/-	Cultivated oats	Kibite and Edney (1992)
1000-grain weight	-/o	Cultivated oats	Holthaus et al. (1996)
Testweight	-	Cultivated oats	Cervantes-Martinez et al. 2002
Testweight	-/+	Cultivated oats	Saastamoinen et al. (1992)
Grain yield	+	Cultivated oats	Saastamoinen et al. (1992)
Grain yield	-/o	Cultivated oats	Kibite and Edney (1992)
Grain yield	+	Cultivated oats	Holthaus et al. (1996)
Grain yield	-/o	Cultivated oats	Cervantes-Martinez et al. 2002
Grain yield	-	Cultivated oats	Baur (1995)
Grain yield	+/-	Cultivated oats	Brunner and Freed (1994)
Kernels/Panicle	o	Cultivated oats	Kibite and Edney (1992)
Heading	+/-	Cultivated oats	Cervantes-Martinez et al. 2002
Heading	-	Cultivated oats	Holthaus et al. (1996)

[1] o: no correlation; +: significant positive; -: significant negative

Unfortunately, compared to the best standard cultivar, there is at least 10 % decrease in yield for the best lines of current population, indicating that these lines need to be crossed further with high yielding breeding lines or cultivars in order to improve the yield.

According to the results of other studies (Cervantes-Martinez et al. 2002, Holthaus et al. 1996, Kibite and Edney 1998, Saastamoinen et al. 1992) there are similar correlations between β-glucan content and agronomic and quality traits (Table 6.1).

Similar as with yield, the correlation coefficients between β-glucan and heading are significantly negative and a consequence of the 10 days earlier heading of the donor for high β-glucan content. Within this population yield is positively correlated with height and heading. In other studies similar or partly contrary ratios were found (Baur 1995, Schipper and Frey 1992).

Oat breeding aims at developing cultivars for different purposes, such as for milling industry or for feeding. There are different demands for both directions of β-glucan and lipid content. For industry purpose, it is expected to have oats containing high β-glucan but rather low lipid content, providing there is no effect on oat flavour (Valentine and Cowan 2004). Because of the negative correlation between β-glucan and lipid content in this population, it should be promising to find lines with the desired trait combinations. Additionally, the positive correlation coefficients between β-glucan and thousand kernel weight, test weight, and protein content, and the negative correlation with husk content make this population valuable for getting lines used as crossing partners for the further development of oat cultivars for milling industry purpose.

6.1.3 ANOVA analysis

The analyses of variance indicated significant environment influences for all characteristics measured (Table 5.3). From present results, soil value points seem to have major effects on yield and β-glucan content. This is based on that the soil in Böhnshausen has a higher value point (74) compared with Granskevitz (54) and Gudow (50); consistently, yield in Böhnshausen had the highest mean values for both years, and β-glucan content (measured by

NIRS) also showed the highest mean value in Böhnshausen.

Low genotype x environment interaction was found for nearly all the traits investigated in present study. This may be due to two reasons: 1.The differences between environments are relatively low; 2.The population investigated in present study was derived from a single BC_2F_2. Thus the lines of the population have similar genetic backgrounds, and these lines may have very similar reactions to the changing environments due to their similar genetic backgrounds.

6.1.4 Evaluation of agronomic values

The selection index is a widespread tool for the rating of breeding lines in plant breeding. The agronomic values of lines of the population 2503 are summarised in Table 5.7. The results displayed a relevant distance to the new cultivars such as 'Aragon'. This is not surprising considering the big distance between the parents of the population and the newer cultivars. However, there are some lines which do have valuable trait combinations. These lines may be useful for further backcrosses.

6.1.5 Marker polymorphism and segregation

Low polymorphism (16.7%) between the two parents was found for 144 SSR primer pairs screened. This low polymorphism is comparable with the results from Li et al. (2000), Pal et al. (2002), and Holland et al. (2001). Li et al. (2000) reported that 62% of their SSR primers revealed polymorphism among *Avena* species, while only 36% among the cultivars. In the study of Pal et al. (2002), 41% of primers revealed polymorphism among 13 *Avena* species, but 14% were polymorphic between the oat cultivars 'Kanota' and 'Ogle'. Similar low polymorphisms were reported by Holland et al. (2001), who reported 21% of SSR markers to be polymorphic among the oat cultivars.

About 450 AFLP polymorphic bands were found between the two parents with 256 primer combinations. The average number of polymorphic bands for each primer combination is 2. This AFLP polymorphism is lower than that of Jin et al. (2000) which had a average polymorphism of 10 bands per primer combination between 'Clintland64' x 'IL86-5698' and 30 polymorphic bands per primer combination between 'Kanota' x 'Ogle'. It is clear that

'Kanota' x 'Ogle' has the highest polymorphism because 'Kanota' and 'Ogle' belong to different *Avena* species, *Avena byzantina* C. Koch and *Avena sativa* L. respectively. The lower polymorphism in present study than that of 'Clintland64' x 'IL86-5698' which both belong to *Avena sativa* L. may be due to different primer combinations. Jin et al. (2000) selected 80 AFLP primer combinations which may had higher polymorphisms for evaluation, while in present study, a total of 256 AFLP primer combinations were used. Huang et al. (2000) reported distinctive differences in the average number of amplified PCR fragments among *Eco*RI + ANN primers combined with 16 *Mse*I + CNN primers in wheat. They concluded that the number of amplified PCR fragments is related directly to *Eco*RI + ANN primer. Similar results were found in present study that different *EcoR*I + ANN primer gave different polymorphisms. Primer E31, E32 and E33 gave fewer polymorphisms, while E34, E37, E38, E39 and E42 gave much higher polymorphisms (data not shown).

Distinct reduction of polymorphism was found when the 24 polymorphic SSR markers and 450 polymorphic AFLP markers were screened in the mapping population. Only 5 (20.8%) of the 24 polymorphic SSR primer pairs gave segregant bands in the mapping population, while 105 (23.3%) for 450 polymorphic AFLP markers. The reduction is reasonable since after 2 backcrosses to the recurrent parent, 75% of the donor parent genome were lost compared with the heterozygous F_1 genome compositions. Thus only 25% of polymorphic markers between the two parents are expected to be segregating in the BC_2F_2 mapping population.

6.1.6 Map development

106 of the 110 segregating markers were mapped into 12 linkage groups, with a total map size of 455.4 cM (Figure 5.2), while 4 markers unlinked. The map is significantly shorter than the published hexaploid oat maps (Table 3.1) for which the total map sizes ranged from 736cM (Groh et al., 2001a) to 2351cM (Jin et al., 2000), and the linkage groups ranged from 27 (Groh et al., 2001a) to 38 (O'Donoughue et al., 1995). The results can again be explained by the BC_2-derived nature of the mapping population.

Significant clustering was found for linkage group 1 and 3 in the map. Especially for linkage group 1, 32 markers clustered within a genetic distance of 15 cM (Figure 5.2). Qi et al. (1998) reported strong clustering of AFLP markers around the centromeric regions in barley. They

explained that the strong clustering of AFLP markers may be due to the centromeric suppression of recombination and the sensitivitiy of the AFLP technique. This is reasonable since the same phenomenon of clustering markers around the centromere was also found in wheat (Devos et al., 1992), barley (Wei et al., 1999) and tomato (Tanksley et al., 1992). In oat, clustering of AFLP markers was reported by Yu and Wise (2000). In contrast, in a report of Jin et al. (2000), well distributed AFLP markers were found in the KO population. Weight et al. (2003) also reported strong clustering of markers on short regions of KO map. Since the centromeric regions haven't been identified in oat maps, it is still not clear whether the clustering markers in oat maps are around the centromeric regions.

Several studies have been conducted on AFLP mapping in both hexaploid (Jin et al., 2000; Groh et al., 2001a; Zhu and Kaeppler, 2003a; De Koeyer et al., 2004) and diploid (Yu and Wise, 2000) oat. Most of these studies combined AFLP markers with anchor RFLP probes or used populations with one shared parent for comparative analysis. Jin et al. (2000) reported that the AFLP polymorphisms in one population were always detected in the second population and were likely linked in the same group. They suggested that most of the co-migrating AFLP bands are homologous and can be used for comparative analysis in different populations. However, they observed that about 11% of the co-migrating AFLP markers were mapped to different linkage groups and concluded the homology of the co-migrating AFLP bands likely depends on the relatedness of the populations. Additionally, since significant structural rearrangements have been found in oat genome (O'Donoughue et al., 1995; Wight et al., 2003), care should be taken when using AFLP markers for comparative analysis.

Though most of the current oat maps were based on RFLP markers, the time and labour consuming character of RFLP analysis limited its applications in present study. In current work, no anchor RFLP probes have been mapped together with AFLP markers. It would be necessary to apply such kind of anchor markers in the population for comparative analysis purposes. One possibility would be to use SSR markers in current population as bridging markers for comparative analysis for the reason that SSR markers are PCR-based, codominant, locus-specific, and evenly distributed along chromosomes. Another subproject of oat SSR markers development is ongoing, and several other groups in Canada and Sweden are also

developing SSR markers. It is possible for oat researchers to get several hundreds or even more of oat SSR markers in near future and these hopeful new marker sources will be great benefit for future comparative analysis.

6.1.7 QTL analysis

6.1.7.1 Method for QTL analysis

Several methods have been used for QTL analysis, including single-point marker analysis (SPA), simple interval mapping (SIM) and composite interval mapping (CIM). In present study the simple interval mapping was used. The principle for single-point marker analysis is to detect the linkage between a single marker and the trait-of-interest by the mean values for different genotypes of the same loci (marker). If a significant difference of the trait mean values was found for different genotypes (classes) of the same marker, it was suggested that the marker was linked with a QTL. However, the single-point marker analysis is not able to give the exact position of a QTL and particularly, wrong positions may result by this method if two QTLs were tightly linked. Composite interval mapping has been widely used for QTL analysis and the method always gives more accurate results. However, for composite interval mapping, one needs to choose suitable markers used as cofactors. This was particularly difficult in the present study due to the clustering of markers in the map used for QTL analysis. Therefore, in present study, the simple interval mapping method was used for QTL analysis.

6.1.7.2 QTL analysis in an advanced backcross population

As a novel molecular breeding method, Advanced Backcross QTL (AB-QTL) mapping was purposed to identify new beneficial alleles from exotic germplasm which are potentially valuable for the improvement of the important agronomic traits of elite breeding lines. Using the AB-QTL approach, it is easier to identify small-effect beneficial alleles and the time needed for line development is reduced since the method combines the identification and transfer of QTLs in the same progress (Tanksley and Nelson, 1996).

In present study, AB-QTL analysis was used to identify and transfer beneficial alleles corresponding to high β-glucan content from the exotic oat line IAH611-447 into the elite oat

cultivar 'Iltis'. The results revealed five QTLs for β-glucan content, which are all caused by favourable alleles from the donor parent IAH611-447. The results indicate that some of the beneficial alleles from the donor parent IAH611-447 were successfully introgressed into the recurrent parent 'Iltis'. However, the five QTLs were all located on the same linkage group, group 1. This result may be due to both the development of the mapping population and the markers used for map construction. Unlike other AB-QTL studies which generally selected more backcrossed individuals for the construction of QTL mapping population in order to get all favourable exotic QTL alleles, the population was derived from only a single BC_2 individual. It is possible that only limited segments were introgressed into the recurrent parent and subsequently, some of the favourable positive alleles from the donor parent were lost. According to the result of the map construction, the linkage group 1 comprised about 33% (36 of 110 markers) of all segregating markers, indicating a larger segment from IAH611-447 was introgressed into 'Iltis'. Since evaluation of β-glucan contents was performed in BC_1F_2 and only limited segments were from IAH611-447, it is possible that the introgressed segment in linkage group 1 had several favourable alleles for high β-glucan contents. Additionally, AFLP markers were used for genetic map construction. Though AFLP is high efficient, there are some disadvantages for the present study. It is not clear for the exact location for each AFLP marker and there is no idea about the distributions of markers over the whole oat genome. Therefore, it is possible that there are some regions where small segments carrying favourable alleles were introgressed but were not able to be detected for the reason that no segregating markers were available in those regions.

6.1.7.3 Consistence of QTLs across environments

In terms of breeding purpose, it is desirable to have QTLs which have consistent effects over environments. Of the 60 QTLs detected, 20 (33%) QTLs were significant in only one environment. The remaining 41 (67%) QTLs were significant in at least two environments. The trait of heading date had the most consistent QTLs across environments, with 2 QTLs significant in all 6 environments, and the remaining one was significant in 3 of the 6 environments. The consistence of 67% QTLs across environments suggests that the interactions by environments for these QTLs are relatively low. And the QTLs detected in different environments all had beneficial alleles from the same parent, indicating the consistent effects of these QTLs.

6.1.7.4 Co-localization of QTLs

For several regions, significant clustering or co-localization of QTLs were found. Especially for linkage group 1, three regions contained QTLs which were significantly associated with 9 to 10 of the 11 traits measured. These three regions of linkage group 1 mapped around position 25 cM which is associated with 10 of 11 traits measured, around position 35 cM which is associated with 9 of 11 traits, and around position 51 cM which is associated with 9 of 11 regions. Similar QTL clustering was found on linkage group 4, for which the region around 9 cM contained QTLs associated with 4 traits; on linkage group 8, for which the region around 6 cM contained QTLs associated with 2 traits; and on linkage group 11, for which the region around 28 cM was associated with 3 traits.

Similar observations were also reported by Fulton et al. (2000) and Frary et al. (2004). Fulton et al. (2000) reported a genome region which is significantly associated with 12 of the 30 traits measured in their study, while Frary et al. (2004) found a marker which is significantly linked with 15 of the 25 traits evaluated. The reason for the QTL clusters may be due to the pleiotropic effects of the same loci, or due to the tight linkage of genes which are associated with those traits. To get sufficient information to determine the exact reason for the clustering QTLs, a larger mapping population and more evenly distributed markers will be needed.

6.1.7.5 QTL comparison and validation

Several studies have been reported for oat QTL analysis (Siripoonwiwat et al., 1996; Ronald et al., 1997; Jin et al., 1998; Kianian et al., 1999, 2000; Barbosa-Neto et al., 2000; De Koeyer et al., 2001; Groh et al, 2001b; Holland et al., 2002; Zhu and Kaeppler, 2003b; Zhu et al., 2003). It is very interesting to find out whether the QTLs identified in present study are the same as those that have been reported by others, or whether they are novel alleles which are not present in the oat elite gene pool so far. However, most of the reported QTLs in oat are based on the KO population or based on RFLP probes which can be joined with the KO map. While in present study, the population investigated has never been reported, and further more, the AFLP markers in present study made it difficult to do comparative analysis. Therefore, development of anchor markers which are segregating both in current mapping population and in the reference KO mapping population would shed some light on the comparison analysis of the identified QTLs. In this respect, those markers appear interesting which have

been identified as being linked to high β-glucan contents by Kianian et al. (2000).

Another question is whether the QTLs identified in present study are small-effect QTLs and can be detected only in the current population. To validate the significant effects of detected QTLs, it is necessary to develop additional populations which have similar genetic backgrounds. Sub-population 2504 is particularly interesting for this purpose since it is derived from the same single BC_1 seed as the sub-population 2503 investigated in present study. It is very probable that these two populations share the same heterozygous genomes in some regions. Therefore, it is expected that part of the QTLs from the sub-population 2503 will also be detected in the sub-population 2504 if the effects of those QTLs are significant enough.

6.2 Genetic analysis of oat powdery-mildew resistance

6.2.1 *Avena macrostachya* is an important genetic resource for oat breeding

Wild species have been widely used as important gene resources for the introgression of interesting traits into various crops. *A. macrostachya* is a wild oat species unique for its perennial and cross-fertilizing characteristics. Several important traits, including winter hardiness (Baum and Rajhathy 1976), resistance to powdery mildew, and BYDV (Hoppe and Pohler 1988) have been found in this species. In unpublished experiments resistances to BYDV and powdery-mildew were confirmed. Two resistance tests with a PAV strain of BYDV and several wild *Avena* species showed that *A. macrostachya* was the only *Avena* species with significant lower virus concentrations in the leaves measured by enzyme-linked immunosorbent assay. Additionally, there was no powdery-mildew infection in any of the *A. macrostachya* plants.

Till now there is only one report (Leggett, 1985) for a successful introgression of useful traits from *A. macrostachya* into cultivated oats, partly because it is very difficult to overcome the sterility of hybrids between *A. macrostachya* and *A. sativa*. The novel gene of *A. macrostachya* investigated in the present study was successfully introgressed into cultivated oat by Pohler and Hoppe (1991), using a bridge cross with *A. maroccana* and an *A. sativa* line with good crossability. After selection in BC_1F_2 for fertility and resistance to powdery-mildew,

a second backcross with susceptible cultivated oats, followed by selfing and selection of valuable plants, stable hexaploid introgression lines with good agronomic value (data not shown) and resistance to powdery-mildew were obtained. According to the patterns of the progenies of the crosses, the normal crossability with *A. sativa*, the fertility of F_1 and selected progenies, and the chromosome numbers, it can be concluded that introgression oat lines with powdery-mildew resistance from *A. macrostachya* were successfully established.

Beside the breeding procedure focused on powdery-mildew resistance, all the progenies of crosses with *A. macrostachya* were examined for tolerance to BYDV and winter hardiness. Despite some small differences no valuable line was found with higher tolerance to BYDV or better winter hardiness than the best standard cultivars. Compared to powdery-mildew resistance, winter hardiness and resistance to BYDV in *A. macrostachya* may be controlled by several genes, which make it more difficult to introgress these traits into cultivated oats.

6.2.2 Inheritance of powdery-mildew resistance

To analyse the inheritance of the powdery-mildew resistance, several segregating populations were developed by crossing two resistant lines, Am27 and Am28, carrying the same resistance gene derived from *A. macrostachya*, with susceptible cultivated oats 'Neklan' and 'Flämingsprofi'. The segregation patterns of all populations confirmed the hypothesis that the resistance is controlled by a single, incompletely dominant gene, tentatively designated *Eg5*. The monofactorial dominant nature of the new source of resistance derived from A. macrostachya makes it useful for resistance breeding in oat.

6.2.3 BSA is powerful for oat marker development

BSA developed by Michelmore et al. (1991) has been used as a fast and technically simple approach to identify markers linked to valuable traits in varies crops. This method is particularly useful for marker development in oat for the reasons that oat is a hexaploid crop with a large genome (11,315 Mb), and the informative makers are relatively limited.

To date, the most complete oat genetic map developed by Wight et al. (2003) has 1166 markers, covering a distance of 1890 cM. However, these markers haven’t been located to 21 oat chromosomes in a single linkage map, and many genetic gaps still need to be filled, which

would be helpful for quick identification of linked markers for important traits and for localization of interesting genes. In this study, without any information about genomic regions for neither the resistance genes nor linked markers, one SSR marker and four STS markers have been easily identified with close genetic distance to the powdery-mildew resistance gene using BSA, confirming the capacity of this method.

6.2.4 SSR as molecular markers for MAS in oat

As PCR-based, highly sensitive and reproducible markers, SSR are easier and faster to use than hybridization-based markers, such as RFLP, for genotyping. SSR could also be used as suitable anchor markers since they are codominant, locus-specific, and evenly distributed along chromosomes. All of these properties make SSR powerful for MAS in oat breeding programmes.

In present study, for the first time, a tightly linked SSR marker, AM102, was identified with a short genetic distance of 0.4 cM and 2 cM to a powdery-mildew resistance gene introgressed in two oat lines Am27 and Am28, respectively. The fragments amplified by the marker AM102 in resistant parent Am28 and susceptible parent 'Flämingsprofi' were 201 bp and 213 bp, respectively. The 0.4 cM genetic distance, together with the 12 bp length polymorphism enable AM102 a reliable and easily detected marker for backcross selection and gene pyramiding to improve powdery-mildew resistances in common oats.

However, the development of SSR markers is expensive and time consuming. One popular way for SSR markers development is to construct enriched libraries and to screen the libraries for useful sequence information in order to design SSR primer sets. Another important way is to use sequences information directly from public databases, for example, EST sequence databases. For oat SSR, only 114 SSR primer sets have been published.

6.2.5 AFLP mapping of *Eg5* and AFLP-derived STS marker development

As rapid and efficient genome-wide screening molecular marker technique, AFLP is sufficient for most cultivated crops to provide genetic maps for MAS purpose. Especially for those crops with large genome, one can relatively easily and quickly proceed to high-density

linkage maps around the economically important genes using AFLP method.

For several reasons it is decided to perform AFLP analysis to find useful markers linked with the resistance gene *Eg5*. The first reason is that there is no information about the genome regions containing powdery-mildew resistance genes and oat is a hexaploid crop with a very large genome. To perform a genome-wide screening for the targeted gene, AFLP is the most efficient method. Secondly, most of current oat maps are based on RFLP markers. RFLP method is both time and labor consuming compared with PCR based markers like SSR and AFLP. As mentioned before, the number of oat SSR markers is quite limited and only one linked SSR marker was found from more than 100 SSR primer pairs. To develop sufficient markers around the resistance gene for MAS program, AFLP would be a good choice.

To perform BSA analysis with AFLP methods, equal amounts of pre-amplified PCR products were used to construct two bulks instead of the original plant DNA. Using pre-amplified DNA for bulk construction should be better for screening since it avoids the possible failure of genomic DNA digestion for some individuals and gives more accurate equal amounts of DNA from each individual. Totally 21 AFLP bands were polymorphic between the two parents, and the two bulks. All of these AFLP markers showed strong linkage with the resistance gene *Eg5*, indicating the success of AFLP method for efficient marker development.

However, AFLP method includes DNA digestion, ligation and two times of PCR amplification. Additionally, AFLP always give too many bands for each reaction and the banding patterns are very complex in hexaploid oat. These disadvantages limited the application of AFLP marker for high-throughput analysis in MAS breeding. For plant breeders, those PCR based markers which are easily handling and give simple banding patterns would be more interesting for practice performing. Therefore, conversion of polymorphic AFLP bands which are tightly linked to the targeted gene into high-throughput PCR based markers is highly desired.

Many studies have been conducted on conversion of AFLP markers into high-throughput easily handling PCR based markers (Negi et al., 2000; Dussle et al., 2002; Sardesai et al., 2002; Stracke et al. 2003; Shirasawa et al., 2004). In current study, 9 AFLP bands which gave

strong polymorphic patterns were selected for STS markers development. The 9 bands responded to 8 AFLP markers, including 1 codominant marker and 7 dominant markers. Finally 4 STS markers were successfully developed from 8 AFLP markers, including 1 codominant marker and 3 dominant markers. The lower efficiency of conversion of dominant AFLP markers into STS markers maybe due to several reasons: Firstly, unlike codominant AFLP marker, it is difficult to confirm the correct clones of the polymorphic AFLP bands. This is because there maybe some AFLP fragments sharing the same size with polymorphic bands but quite different sequences. While for codominant AFLP markers which are normally caused by insertions/deletions (indels), two bands can be cloned and the correct clones can be easily confirmed by sequence comparison of the two alleles. Secondly, some of dominant AFLP markers are caused by small nucleotide differences in restriction enzyme cutting sites, in current case, for example, differences in *EcoR*I or *Mse*I sites. For this kind of polymorphisms, even correct AFLP bands were cloned, it is still difficult to reveal the same polymorphisms by a simple PCR since there is no size differences for the corresponding AFLP bands. Thirdly, DNA methylation in enzyme cutting sites may be the reason. Shirasawa et al. (2004) failed to converse 7 dominant AFLP markers into sequence-specific markers because the nucleotide sequences of the genome regions covering the polymorphic AFLP markers were the same between the cultivars they investigated. They explained that different band patterns of the same nucleotide sequences in the AFLP analysis would be DNA methylation in one of the cultivars.

For those interested dominant AFLP markers, additional CAPS analysis with different restriction enzymes may be able to reveal polymorphisms which are not able to be detected by a simple PCR. Interestingly, Brugmans et al. (2003) reported a new procedure for the identification of the SNP responsible for the AFLP markers and with their approach, a high success rate for the conversion of AFLP markers into locus-specific markers was obtained.

6.2.6 Mapping of resistance gene *Eg5*

Pal et al. (2002) assigned AM102 to linkage group 22 in a 'Kanota' x 'Ogle' mapping population (O'Donoughue et al., 1995). The results confirmed the location of AM102 in detail and located AM102 between RFLP marker cdo419 and cdo484a in the KO22_44+18 linkage

group(Wight et al. 2003), with a genetic distance of 1 cM to cdo419, and 3 cM to cdo484a (Figure 5.10C). Several QTLs for resistance to crown rust and BYD have been located in these regions (Zhu et al., 2003) and, recently, a resistance gene analog (RGA) marker was also positioned on this linkage group (Irigoyen et al., 2004). Interestingly, Wight et al. (2004) mapped the crown rust resistance gene *Pc48* in the KO group 22_44+18, giving this region special interests for resistance-gene investigation.

However, since only one linked marker, AM102, was joined into the KO group 22_44+18, it is still difficult to give the precise position of the powdery-mildew resistance gene *Eg5* in the KO map. Moreover, for both resistant parents Am27 and Am28 used to produce the mapping populations, the sizes of introgressed segments containing the resistance gene *Eg5* from *A. macrostachya* are not clear yet. Thus, development of further closely linked anchor markers around the resistance gene would be necessary to saturate this region and to localize the gene correctly in the KO map, and further more, to give detailed information about the sizes of the introgression segments from the wild species *A. macrostachya* in two resistant parents Am27 and Am28.

The markers identified in this study, particularly the two codominant markers AM102 and ASE41M56, should be useful for both marker-assisted selection breeding purpose and further study of oat genome regions containing different powdery-mildew resistance genes.

7 Conclusions and outlooks

7.1 Oat AB-QTL analysis

AB-QTL analysis was performed in a BC_2F_2 population derived from a cross of 'Iltis' x IAH611-447. 11 traits were evaluated for 98 $BC_2F_{2:5-6}$ lines at 3 locations in two years. ANOVA analysis revealed significant differences between environments and among population lines for all the traits evaluated. Consistent correlation coefficients between β-glucan content and other traits were found in all environments. 110 markers, including 105 AFLP and 5 SSR markers were identified for map construction. A linkage map of 455cM with 12 linkage groups was developed for the BC_2F_2 population. Significant marker clustering was found for 2 linkage groups in current linkage map. QTL analysis was conducted using simple interval mapping (SIM). A total of 60 significant QTLs were detected for all 11 traits. For β-glucan content, 4 QTLs were detected and all the 4 QTLs have favorable alleles from the donor parent IAH611-447, explaining the phenotypic variation from 11.9% to 44.2%.

Several results of the present study need to be improved and confirmed by future research. These include population development, map construction, QTL validation and comparison.

1. The population investigated in the present study was derived from a single BC_2 plant. This population contains only limited genomic regions introgressed from the donor parent and some valuable alleles may be lost. Therefore, selecting more individuals for crossing to develop a population containing more favorable alleles from exotic germplasms will be useful.
2. The linkage map used for QTL mapping in present study was mainly developed by dominant AFLP markers. This kind of marker is less suitable for MAS breeding purpose and is difficult for comparative analysis. Additionally, significant marker clustering was found in current linkage map. The problems may be resolved if suitable codominant anchor markers, such as SSR markers, can be applied in current population.
3. It is not sure whether the QTLs identified in the present study are novel alleles or they are alleles already available. It is also not sure whether the QTLs have significant effects which can be also detected in other populations. And it is not clear about the reason for the significant QTL clustering found in present study. Therefore, additional evenly distributed

anchor markers and additional bigger populations need to be investigated for the comparison and validation of the QTLs detected in present study.

7.2 Genetic study of an introgressed powdery-mildew resistance gene derived from *Avena macrostachya* in oat (*Avena sativa*)

For the first time, an introgressed powdery-mildew resistance gene derived from *Avena macrostachya* was investigated in hexaploid oat. Genetic analysis revealed that the resistance is controlled by an incompletely dominant gene, tentatively designated *Eg5*. Molecular marker analysis was conducted using bulked segregant analysis in two segregating $F_{2:3}$ populations. One codominant simple sequence repeat (SSR) marker, AM102, was identified tightly linked to *Eg5* with a genetic distance of 0.4 cM and 2 cM in two populations. 4 AFLP-derived PCR-based markers were also successfully developed. Linkage analysis revealed all these molecular markers were tightly linked with the resistance gene in both segregating populations and the SSR marker AM102 was also mapped on the KO-22_44+18 linkage group.

The new resistance source derived from *A. macrostachya*, together with the tightly linked PCR-based markers identified here, would be beneficial for gene pyramiding to improve powdery-mildew resistances in future oat breeding programs.

However, more efforts need to be conducted in order to study the following topics which haven't been resolved in present study.

1. The exact location of *Eg5*. Since only one resistance marker has been mapped on the KO-22_44+18 linkage group, the exact location of *Eg5* is still not clear, especially considering of significant structure rearrangements found in oat genome (O'Donoughue et al., 1995; Wight et al., 2003).
2. The relationship between *Eg5* and the other powdery-mildew resistance genes. Since quite limited research was reported on oat powdery-mildew resistance genes, and no linkage analysis has been done on those reported genes, it is not sure whether the resistance gene

derived from wild species *A. macrostachya* is really a ‘novel’ gene, thus there is no idea about the linkage information between these genes.

3. Linkage drag analysis. Linkage drag is always a serious problem during the introgression of exotic genes. In the current study, there is no information on the size of introgressed fragment from *A. macrostachya*.

The problems mentioned above may be resolved when more markers around the resistance gene are available and more crosses between current resistant lines with other lines containing different powdery-mildew resistance genes are made.

8 References

Akkaya, M. S., Bhagwat, A. A. and Cregan, P. B., (1992). Length polymorphisms of simple sequence repeat DNA in soybean. Genetics **132**: 1131-1139.

Aung, T., Thomas, H. and Jones, I.T. (1977). The transfer of the gene for mildew resistance from *Avena barbata* (4x) into the cultivated oat *A. sativa* by an induced translocation. Euphytica **26**: 623-632.

Barbosa-Neto, J. F., Siripoonwiwat, W., O'Donoughue, L. S., Gray, S. M., Smith, D. M., Kolb, F. L., Gourmet, C., Brown, C. M., and Sorrells, M. E. (2000). Chromosomal regions associated with barley yellow dwarf virus resistance in oat. Euphytica **114**: 67-76.

Baum, B. R., and Rajhathy, T. (1976). A study of *Avena macrostachya*. Can J Bot **54**: 2434-2439.

Baur, S. K. (1995). Untersuchungen zur Vererbung des (1-3),(1-4)-β-D-Glucangehaltes in der Karyopse von Hafer und Sommergerstengenotypen unter Berücksichtigung methodischer und ertragsanalytischer Fragen, Diss. CAU Kiel.

Bernacchi, D., Beck-Bunn, T., Eshed, Y., Lopez, J., Petiard, V., Uhlig, J., Zamir, D., and Tanksley, S. D. (1998). Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from Lycopersicon hirsutum. Theor Appl Genet **97**: 381-397.

Bernatzky, R. and Tanksley, S. D. (1986). Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics **112**: 887-898.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. **32**: 314-331.

Brugmans, B., van der Hulst, R. G. M., Visser, R. G. F., Lindhout, P. and van Eck, H. J. (2003). A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Res. **31**: e55.

Brunner, B. R. and Freed, R.D. (1994). Oat grain β-glucan content as affected by nitrogen level, location, and year. Crop Sci **34**: 473-476.

Burke, D., Kaufman, P., McNeil, M. and Albersheim, P. (1974). The structure of plant cell walls. VI. A survey of the walls of suspension-cultured monocots. Plant Physiol **54**: 109-115.

Bush, A. L. and Wise, R. P. (1998). High-resolution mapping adjacent to the *Pc71* crown-rust resistance locus in hexaploid oat. Mol Breed **4**: 13-21.

Cervantes-Martinez, C. T., K.J. Frey, P.J. White, D.M. Wesenberg, J.B. Holland (2001). Selection for greater β-glucan content in oat grain. Crop Sci. **41**: 1085-1091.

Cervantes-Martinez, C. T., K.J. Frey, P.J. White, D.M. Wesenberg, J.B. Holland (2002). Correlated responses to selection of greater β-glucan content in two oat populations. Crop Sci.

42: 730-738.

Cheng, D. W., K. C. Armstrong, et al. (2002). Genetic and physical mapping of *Lrk10*-like receptor kinase sequences in hexaploid oat (*Avena sativa* L.). Genome **45**: 100-109.

Chong, J., E. Reimer, et al. (2004). Development of sequence-characterized amplified region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. Canadian Journal of Plant Pathology **26**: 89-96.

Colleoni-Sirghie, M., B. D. Fulton, P.J. White (2003). Structural features of water soluble (1,3) (1,4)-beta-D-glucans from high-beta-glucan and traditional oat lines. Carbohydrate Polymers **54**: 237-249.

Condit, R. and Hubbell, S. P. (1991). Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome **34**: 66-71.

De Koeyer, D. L., Phillips, R. L. and Stuthman, D. D. (2001). Allelic shifts and quantitative trait loci in a recurrent selection population of oat. Crop Sci **41**: 1228_1234.

De Koeyer, D. L., Tinker, N. A., Wight, C. P., Deyl, J., Burrows, V. D., O'Donoughue, L. S., Lybaert, A., Molnar, S. J., Armstrong, K. C., Fedak, G., Wesenberg, D. M., Rossnagel, B. G. and McElroy, A. R. (2004). A molecular linkage map with associated QTLs from a hulless × covered spring oat population. Theor Appl Genet **108**: 1285-1298.

Devos, K. M., Atkinson, M. D., Chinoy, C. N., Liu, C. J. and Gale, M. D. (1992). RFLP-based genetic map of the homoeologous group-3 chromosomes of wheat and rye. Theor Appl Genet **83**: 931-939.

Dussle, C. M., Quint, M., Xu, M. L., Melchinger, A. E. and Lübberstedt, T. (2002). Conversion of AFLP fragments tightly linked to SCMV resistance genes Scmv1 and Scmv2 into simple PCR-based markers. Theor Appl Genet **105**: 1190-1195.

Estrada, A., van Kessel, A. and Laarveld, B. (1997). Immunomodulatory activities of oat beta-glucan in vitro and in vivo. Microbiol Immunol **41**(12): 991-8.

Frary, A., Fulton, T. M., Zamir, D. and Tanksley, S. D. (2004). Advanced backcross QTL analysis of a Lycopersicon esculentum × L. pennellii cross and identification of possible orthologs in the Solanaceae. Theor Appl Genet **108**: 485-496.

Fulton, T. M., Grandillo, S., Beck-Bunn, T., Fridman, E., Frampton, A., Lopez, J., Petiard, V., Uhlig, J., Zamir, D. and Tanksley, S. D. (2000). Advanced backcross QTL analysis of a Lycopersicon esculentum × Lycopersicon parviflorum cross. Theor Appl Genet **100**: 1025-1042.

Groh, S., Zacharias, A., Kianian, S. F., Penner, G. A., Chong, J., Rines, H. W. and Phillips, R. L. (2001a). Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet **102**: 876-884.

Groh, S., Kianian, S. F., Phillips, R. L., Rines, H. W., Stuthman, D. D., Wesenberg, D. M. and

Fulcher, R. G. (2001b). Analysis of factors influencing milling yield and their association to other traits by QTL analysis in two hexaploid oat populations. Theor Appl Genet **103**: 9-18.

Hahn, J. D., Chung, T. K. and Baker, D. H. (1990). Nutritive value of oat flour and oat bran. J. Anim. Sci. **68**: 4253-4260.

Hayes, J. D. and Jones, I. T. (1966). Variation in the pathogenicity of *Erysiphe graminis* D.C. f. sp. *avenae*, and its relation to the development of mildew resistant oat cultivars. Euphytica **15**: 80-86.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A. and Nienhuis, J. (1986). Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet **72**(761-769).

Herrmann, M., and Roderick, H. W. (1996). Characterisation of new oat germplasm for resistance to powdery mildew. Euphytica **89**: 405-410.

Holland, J. B., Helland, S. J., Sharopova, N. and Rhyne, D. C. (2001). Polymorphism of PCR-based markers targeting exons, introns, promoter regions, and SSRs in maize and introns and repeat sequences in oat. Genome **44**: 1065-1076.

Holland, J. B., Portyanko, V. A., Hoffman, D. L. and Lee, M. (2002). Genomic regions controlling vernalization and photoperiod responses in oat. Theor Appl Genet **105**: 113_126.

Holthaus, J. F., Holland, J. B., White, P. J. and Frey, K. J. (1996). Inheritance of β-glucan content of oat grain. Crop Sci **36**: 567-572.

Hoppe, H.-D. and Pohler, W. (1988). Successful hybridisation between *Avena prostrata* and *A. macrostachya*. Cereal Res Commun **16**(231-235).

Hoppe, H.-D. and Kummer, M. (1991). New productive hexaploid derivatives after introgression from *A. pilosa* features. Vortr. Pflanzenzüchtg. **20**: 56-61.

Hsam, S. L. K., Peters, N., Paderina, E. V., Felsenstein, F., Oppitz, K. and Zeller, F. J. (1997). Genetic studies of powdery mildew resistance in common oat (*Avena sativa* L.) I. Cultivars and breeding lines grown in Western Europe and North America. Euphytica **96**: 421-427.

Hsam, S. L. K. and Zeller, F. J. (1998). Chromosomal location of genes for resistance to powdery mildew in cultivated oat (*Avena sativa* L.). 1. Gene *Eg-3* in the cultivar 'Mostyn'. Plant Breed. **117**: 177-178.

Huang, X. Q., Zeller, F. J., Hsam, S. L. K., Wenzel, G. and Mohler, V. (2000). Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome **43**: 298-305.

Huang, X. Q., Cöster, H., Ganal, M. W. and Röder, M. S. (2003). Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (Triticum aestivum L.). Theor Appl Genet **106**: 1379-1389.

Humphreys, D. G. and Mather, D. E. (1996). Heritability of *β*-glucan, groat-percentage, and crown rust resistance in two oat crosses. Euphytica **91**: 359-364.

Irigoyen, M. L., Loarce, Y., Fominaya, A.and Ferrer, E. (2004). Isolation and mapping of resistance gene analogs from the *Avena strigosa* genome. Theor Appl Genet **109**: 713-24.

Jin, H., Domier, L. L., Kolb, F. L. and Brown, C. M. (1998). Identification of quantitative loci for tolerance to barley yellow dwarf virus in oat. Phytopathology **88**: 410-415.

Jin, H., Domier, L. L., Shen, X. and Kolb, F. L. (2000). Combined AFLP and RFLP mapping in two hexaploid oat recombinant inbred populations. Genome **43**: 94-101.

Jones, I. T. (1978). Components of adult plant resistance to powdery mildew (Erysiphe graminis f.sp. avenae) in oats. Annals of applied biology **90**: 233-239.

Jones, I. T. (1983). Transgressive segregation for enhanced level of adult plant resistance to mildew in the oat cross Mostyn x Maldwyn. Euphytica **32**: 499-503.

Khush, G. S., Singh, R. J., Sur, S. C. and Librojo, A. L. (1984). Primary trisomics of rice Origin, morphology, cytology and use in linkage mapping. Genetics **107**: 141-163.

Kianian, S. F., Egli, M. A., Phillips, R. L., Rines, H. W., Somers, D. A., Gengenbach, B. G., Webster, F. H., Livingston, S. M., Groh, S., O'Donoughue, L. S., Sorrells, M. E., Wesenberg, D. M., Stuthman, D. D. and Fulcher, R. G (1999). Association of a major groat oil content QTL and an acetyl-CoA carboxylase gene in oat. Theor Appl Genet **98**: 884-894.

Kianian, S. F., Phillips, R. L., Rines, H. W., Fulcher, R. G., Webster, F. H. and Stuthman, D. D. (2000). Quantitative trait loci influencing *β*-glucan content in oat (*Avena sativa*, 2n=6x=42). Theor Appl Genet **101**: 1039-1048.

Kibite, S. and Edney, M. J. (1998). The inheritance of *β*-glucan concentration in three oat (*Avena sativa* L.) crosses. Can. J. Plant Sci. **78**: 245-250.

Lawes, D. A. and Hayes, J. D. (1965). The effect of mildew (Erysiphe graminis f.sp. avenae) on spring oats. Plant pathology **14**: 125-128.

Leggett, J. M. (1985). Interspecific hybrids involving the perennial oat species *Avena macrostachya*. Can J Genet Cytol **27**: 29-32.

Leggett, J. M. and Thomas, H. (1995). Oat evolution and cytogenetics. p. 121-149. In R.W. Welch (ed.) The oat crop: Production and utilization. 1st ed. Chapman and Hall, London.

Leggett, J. M. and Markhand, S. M. (1995). The genomic identification of some monosomics of *Avena sativa* L. cv. Sun II using GISH. Genome **38**: 747-751.

Li, C. D., Rossnagel, B. G. and Scoles, G. J. (2000). The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet **101**: 1259-1268.

Lim, H. S., White, P. J. and Frey, K. J. (1992). Genotypic effects on *β*-glucan content of oat

lines grown in two consecutive years. Cereal Chem. **69**: 262-265.

Linares, C., Gonzalez, J., Ferrer, E. and Fominaya, A. (1996). The use of double in-situ hybridization to physically map the positions of 5S rDNA genes in relation to the chromosomal location of 18S-5.8S-26S rDNA and a C genome specific DNA sequence in the genus *Avena*. Genome **39**: 535-542.

Linares, C., Ferre, E. and Fominaya, A. (1998). Discrimination of the closely related A and D genomes of the hexaploid oat *Avena sativa* L. Proc Natl Acad Sci USA **95**: 12450-12455.

Markert, C. L. and Moller, F. (1959). Multiple forms of enzyme: tissues, ontogenetic, and species specific patterns. Proc Natl Acad Sci USA **45**: 753-763.

McCleary, B. V. and Glennie-Holmes, M. (1985). Enzymatic quantification of (1 3), (1 4)-*β*-glucan in barley and malt. J. Inst. Brew. **91**: 285-295.

Michelmore, R. W., Paran, I., Kesseli, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA **88**: 9828-9832.

Miller, S. S., Wood, P. J., Pietrzak, L. N. and Fulcher, R. G. (1993a). Mixed linkage *β*-glucan, protein content, and kernel weight in *Avena* species. Cereal Chem. **70**: 231-233.

Miller, S. S., Vincent, D. J., Weisz, J. and Fulcher, R. G. (1993b). *β*-glucan: An evaluation of eastern Canadian cultivars and unregistered lines. Can. J. Plant Sci. **73**: 429-436.

Moncada, P., Martínez, C. P., Borrero, J., Chatel, M., Gauch Jr, H., Guimaraes, E., Tohme, J. and McCouch, S. R. (2001). Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theor Appl Genet **102**: 41-52.

Negi, M. S., Devic, M., Delseny, M. and Lakshmikumaran, M. (2000). Identification of AFLP fragments linked to seed coat colour in Brassica juncea and conversion to a SCAR marker for rapid selection. Theor Appl Genet **101**: 146-152.

O'Donoughue, L. S., Kianian, S. F., Rayapati, P. J., Penner, G. A., Sorrells, M. E., Tanksley, S. D., Phillips, R. L., Rines, H. W., Lee, M., Fedak, G., Molnar, S. J., Ho§man, D., Salas, C. A., Wu, B., Autrique, E. and Van Deynze, A. (1995). A molecular linkage map of cultivated oat. Genome **38**: 368-380.

O'Donoughue, L. S., Chong, J., Wight, C. P., Fedak, G. and Molnar, S.J. (1996). Localization of stem rust resistance genes and associated molecular markers in cultivates oat. Phytopathology **86**(719-727).

Pal, N., Sandhu, J. S., Domier, L. L. and Kolb, F. L. (2002). Development and Characterization of Microsatellite and RFLP-Derived PCR Markers in Oat. Crop Sci **42**: 912-918.

Paran, I. and Michelmore, R. W. (1993). Development of reliable PCR-based markers linked

to downy mildew resistance genes in lettuce. Theor Appl Genet **85**: 985-993.

Paterson, A. H., Lander, E. S., Hewitt, J. D., Peterson, S., Lincoln, S. E. and Tanksley, S. D. (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature **335**: 721-726.

Payne, P. I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Ann. Rev. Plant. Physiol. **38**: 141-153.

Penner, G. A., Chong, J. Levesque-Lemay, M., Molnar, S. J. and Fedak, G. (1993a). Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet **85**: 702-705.

Penner, G. A., Chong, J., Wight, C. P., Molnar, S. J. and Fedak, G. (1993b). Identification of a RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome **36**: 818-820

Pérez, T., Albornoz, J. and Dominguez, A. (1998). An evaluation of RAPD fragment reproducibility and nature. Mol. Ecol. **7**: 1347-1357.

Peterson, D. M. (1991). Genotype and environment effects on oat beta-glucan concentration. Crop Sci. **31**: 1517-1520.

Peterson, D. M. (1992). Oat *β*-glucan and tocols. A. R. Barr (eds.), Proceedings of the 4th International oat conference, Vol. 1, The changing role of oats in human and animal nutrition, Adelaide, Australia, 19-24.

Pillen, K., Zacharias, A. and Léon, J. (2003). Advanced backcross QTL analysis in barley (Hordeum vulgare L.). Theor Appl Genet **107**: 340-352.

Pohler, W. and Hoppe, H.-D. (1991). *Avena macrostachya* - a potential gene source for oat breeding. Vortr. Pflanzenzüchtg. **20**: 66-71.

Portyanko, V. A., Hoffman, D. L., Lee, M. and Holland, J. B. (2001). A linkage map of hexaploid oat based on grass anchor DNA clones and its relationship to other oat maps. Genome **44**: 249-265.

Qi, X., Stam, P. and Lindhout, P. (1998). Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet **96**: 376 - 384.

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.-H., Leroy, P. and Ganal, M. W. (1998). A microsatellite map of wheat. Genetics **149**: 2007-2023.

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

Ranhotra, G. S. and Gelroth, J. A. (1995). Food uses of oats. p. 409-430. In R.W. Welch (ed.) The oat crop: Production and utilization. 1st ed. Chapman and Hall, London.

Roderick, H. W., Jones, E. R. L. and Sebesta, J. (2000). Resistance to oat powdery mildew in Britain and Europe: a review. Ann. appl. Biol. **136**: 85-91.

Ronald, P. S., Penner, G. A., Brown, P. A. and Brule-Babe, A. (1997). Identification of RAPD markers for percent hull in oat. Genome **40**: 873-878.

Rooney, W. L., Rines, H., W. and Phillips, R. L. (1994). Identification of RFLP markers linked to crown rust resistance genes *Pc91* and *Pc92* in oat. Crop Sci **34**: 940-944.

Saastamoinen, M., Plaami, S. and Kumpulainen, J. (1992). Genetic and environmental variation in _-glucan content of oats cultivated or tested in Finland. J. Cereal Sci. **16**(279-290).
Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R.A. and Ribosomal, A. R. W. (1984). DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA **81**: 8014-8018.

Sardesai, N., Kumar, A., Rajyashri, R., Nair, S. and Mohan, M. (2002). Identification and mapping of an AFLP marker linked to *Gm7*, a gall midge resistance gene and its conversion to a SCAR marker for its utility in marker aided selection in rice. Theor Appl Genet **105**: 691-698.

Schipper, H. and Frey, K. J. (1992). Responses in agronomic traits associated with three recurrent selection regimes for groat-oil content in oat. Plant Breed. **108**: 302-313.

Sebesta, J., Roderick, H. W., Chong, J. and Herder, D. E. (1993). The oat line Pc54 as a source of resistance to crown rust, stem rust and powdery mildew in Europe. Euphytica **71**: 91-97.

Shinnick, F. L., Mathews, R. and Ink, S. (1991). Serum cholesterol reduction by oats and other fiber sources. Cereal Foods World **36**: 815-821.

Shirasawa, K., Kishitani, S. and Nishio, T. (2004). Conversion of AFLP markers to sequence-specific markers for closely related lines in rice by use of the rice genome sequence. Mol Breed **14**: 283_292.

Siripoonwiwat, W., O'Donoughue, L. S., Wesenberg, D., Hoffman, D. L., Barbosa-Neto, J. F. and Sorrells, M. E. (1996). Chromosomal regions associated with quantitative traits in oat. J. Quant. Trait Loci **2**: 1-17.

Stracke, S., Schilling, A. G., Forster, J., Weiss, C., Glass, C., Miedaner, T. and Geiger, H. H. (2003). Development of PCR-based markers linked to dominant genes for male-fertility restoration in Pampa CMS of rye (Secale cereale L.). Theor Appl Genet **106**: 1184-1190.

Tanksley, S. D., Ganal, M. W., Prince, J. P., De Vicente, M. C., Bonierbale, M. W., Broun, P., Fulton, T. M., Giovannoni, J. J., Grandillo, S., Martin, G. B., Messeguer, R., Miller, J. C., Miller, L., Paterson, A. H., Pineda, O., Röder, M. S., Wing, R. A., Wu, W, and Young, N. D. (1992). High-density molecular linkage maps of the tomato and potato genomes. Genetics **132**: 1141-1160.

Tanksley, S. D. and Nelson, J. C. (1996). Advanced backcross QTL analysis: a method for the

simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet **92**: 191-203.

Tanksley, S. D., Grandillo, S., Fulton, T. M., Zamir, D., Eshed, T., Petiard, V., Lopez, J. and Beck-Bunn, T. (1996). Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative L. pimpinellifolium. Theor Appl Genet **92**: 213-224.

Tautz, D. and Renz, M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. **12**: 4127-4138.

Thomas, H., Powell, W. and Aung, T. (1980). Interferring with regular meiotic behaviour in *Avena sativa* as a method of incorporating the gene for mildew resistance from *A. barbata*. Euphytica **29**: 635-640.

Utz, H. F. (2000). PLABSTAT: A Computer Program for Statistical Analysis of Plant Breeding Experiments, Version 2N. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Utz, H. F. and MELCHINGER, A. E. (1996). PLABQTL: a program for composite interval mapping of QTL. J. Quant. Trait Loci 2(1).

Valentine, J. and Cowan, S. (2004). New directions in breeding for high quality oats. P.Peltonen-Sainio and M. Topi-Hulmi (eds.) Proceedings of the 7th. International oat conference, Helsinki, 45-50.

Van Horn, L., Emidy, L. A., Liu, K. A., Liao, Y. L., Ballew, C., King, J. and Stamler, J. (1988). Serum lipid response to a fat-modified, oatmeal-enhanced diet. Prev. Med. **17**: 377-386.

Van Ooijen J. W., Voorrips, R. E. (2001) JoinMap version 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van der, L. T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kupier, M. and Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407-4414.

Wei, F., Gobelman-Werner, K., Morroll, S. M., Kurth, J., Mao, L., Wing, R., Leister, D., Schulze-Lefert, P. and Wise, R. P. (1999). The Mla (Powdery Mildew) Resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics **153**: 1929-1948.

Weibull, J. (1986). Screening for resistance against Rhopalosiphum padi (L.). I. *Avena* species and breeding lines. Euphytica **35**: 993-999.

Welch, R. W. and Lloyd, J. D. (1989). kernel (1 3),(1 4)-β-D-glucan content of oat genotypes. J. Cereal Sci. **9**: 35-40.

Welch, R. W., Leggett, J. M. and Lloyd, J. D. (1991). Variation in kernel (1 3), (1

4)-*β*-D-glucan content of oat cultivars and wild *Avena* species and its relationship to other characteristics. J. Cereal Sci. **13**: 173-178.

Welch, R. W. (1995). The chemical composition of oats. p. 304-305. In R.W. Welch (ed.) The oat crop: Production and utilization. 1st ed. Chapman and Hall, London.

Welch, R. W., Brown, J. C. W. and Leggett, J. M. (2000). Interspecific and Intraspecific Variation in Grain and Groat Characteristics of Wild Oat (*Avena*) Species: Very High Groat (1 3), (1 4)-*β*-D-glucan in an *Avena atlantica* Genotype. J. Cereal Sci. **31**: 273-279.

Wight, C. P., Tinker, N. A., Kianian, S. F., Sorrells, M. E., O'Donoughue, L. S., Hoffman, D. L., Groh, S., Scoles, G. J., Li, C. D., Webster, F. H., Phillips, R. L., Rines, H. W., Livingston, S. M., Armstrong, K. C., Fedak, G. and Molnar, S. J. (2003). A molecular marker map in 'Kanota' × 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome **48**: 28-47

Wight, C. P., O'Donoughue, L. S., Chong, J., Tinker, N. A. and Molnar, S. J. (2004). Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes Pc38, Pc39, and Pc48 in cultivated oat (*Avena sativa* L.). Mol. Breed. **14**: 349 - 361.

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

Xiao, J., Li, J., Grandillo, S., Ahn, S. N., Yuan, L., Tanksley, S. D. and McCouch, S. R. (1998). Identification of Trait-Improving Quantitative Trait Loci Alleles From a Wild Rice Relative, Oryza rufipogon. Genetics **150**: 899-909.

Yu, G.-X. and Wise, R. P. (2000). An anchored AFLP- and retrotransposon-based map of diploid *Avena*. Genome **43**: 736-749.

Zhu, S., Kolb, F. L. and Kaeppler, H. F. (2003). Molecular Mapping of genomic regions underlying barley yellow dwarf tolerance in cultivated oat (*Avena sativa* L.). Theor Appl Genet **106**: 1300-1306.

Zhu, S. and Kaeppler, H. F. (2003a). A genetic linkage map for hexaploid, cultivated oat (*Avena sativa* L.) based on an intraspecific cross 'Ogle/MAM17-5'. Theor Appl Genet **107**: 26-35.

Zhu, S. and Kaeppler, H. F. (2003b). Identification of quantitative trait loci for resistance to crown rust in oat line MAM17–5. Crop Sci **43**: 358-366.

Acknowledgements

I would like to show my sincere appreciation to Dr. M. Herrmann and Prof. Dr. P. Wehling, Institut für landwirtschaftliche Kulturen, Bundesanstalt für Züchtungsforschung an Kulturpflanzen, for giving me the opportunity to work on this interesting project. Without their countless discussions and continuing supports, this work would not be able to be finished. I wish them and their research all the best.

Special thanks must go to Prof. Dr. W. E. Weber, Institut für Pflanzenzüchtung und Pflanzenschutz, Martin-Luther-Universität Halle-Wittenberg, for accepting me as a Ph.D. student. I also thank him for his valuable suggestions and critical reading of this dissertation.

I do appreciate Dr. St. Beuch, Nordsaat Saatzucht GmbH, for his attendance on trials evaluation and for his critical review of this dissertation. It is always a wonderful time to be with him. And I must extend my thanks to Dr. S. Maak, Institut für Pflanzenzüchtung und Pflanzenschutz, Martin-Luther-Universität Halle-Wittenberg, for his careful review and valuable suggestions of this manuscript.

One of the reasons that the last three years have gone so quickly is the colleagues of Institut für landwirtschaftliche Kulturen, Bundesanstalt für Züchtungsforschung an Kulturpflanzen (BAZ) I've had the pleasure to work with. Especially I would like to thank Dr. B. Hackauf, Dr. B. Ruge for their countless assistance during my lab work at Groß Lüsewitz.

I also want to thank Prof. Dr. Yueming Yan, Life College, Capital Normal University, China, for his encouragement and support during my PhD work.

My great gratitude should go to my father, my mother, and my brother. They are always behind and with me.

My biggest appreciation is for my wife, Yulan Xiong. Without her love, her encouragement and her support, I would not be able to write these words here.

Curriculum vitae

Name:	Jianzhong Yu
Date and place of birth:	21th January 1976, born in QiChun, Hubei province, China
Sex:	Male
Nationality:	Chinese

Education:

09.1982-07.1987	Primary school in QiChun, Hubei province, China
09.1987-07.1995	Junior and high middle school in QiChun, Hubei province, China
09.1995-07.1999	Department of Biology, Capital Normal University, China Majored in Biology, B.Sc. degree
09.1999-07.2002	Department of Biology, Capital Normal University, China Majored in Molecular Genetics, M.Sc. degree

Employment:

08.2002-01.2003	Lab of Functional Genomics, Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, China
Since 02.2003	PhD student in Institute of Agricultural Crops, Federal Centre for Breeding Research on Cultivated Plants, Groß Lüsewitz, Germany

E R K L Ä R U N G

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

Hiermit erkläre ich, dass diese Arbeit von mir angefertigt wurde. Es wurden keine anderen als die in der Literatur zitierten Hilfsmittel benutzt.

Desweiteren erkläre ich, daß keine Strafverfahren gegen mich anhängig sind.

Halle/Saale, den 22.06.2005

Jianzhong Yu