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Quantitative Trait Analysis of Heterosis-relevant Loci using Molecular Markers in Oilseed Rape (*Brassica napus* L.)

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List of Abbreviations and Symbols

AFLP	Amplified Fragment Length Polymorphism
BC	back cross
cDNA	complementary DNA
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DH	doubled-haploid
DNA	deoxyribonucleic acid
F1, F2, F3	filius 1 (1st generation after crossing), and so on
KWS	Kleinwanzlebener Saatzucht AG (former name)
MCMC	Markov-chain Monte Carlo method
MPH	mid-parent heterosis
MSL	Male-Sterile Lembke
P	probability
P1, P2	first parent, second parent
QTL	quantitative trait loci
r	coefficient of correlation
RIL	recombinant inbred lines
RNA	ribonucleic acid
SAGE	Serial Analysis of Gene Expression
SLA	specific leaf area (area per dry mass of leaf)
SLW	specific leaf weight (dry mass per unit area of leaf)
SSR	simple-sequence repeats
TSM	thousand seed mass

1 INTRODUCTION

1.1 Introduction and literature review

Oilseed rape (*Brassica napus* ssp. *napus*) is the most important oilseed crop in Europe and the second most important worldwide after soybean. In recent decades demand for rapeseed oil as a nutritional, industrial and fuel oil has risen dramatically, meaning that increasing the seed yield has a high priority for breeding of new varieties. Since the discovery and development of male-sterile systems suitable for hybrid oilseed rape production, hybrid varieties are today gaining an increasing market share. As an illustration, from 17 hybrid cultivars among the 62 approved German '00' (low glucosinolate, zero erucic acid) winter oilseed rape cultivars listed by *Bundessortenamt*, the German Variety Registration Office, in 2008 (Bundessortenamt 2008), more than 60% of the winter oilseed rape crop in 2007/08 was made up of hybrids (Rapool Online 2009). Since the 2003/04 growing season hybrid cultivars have dominated Germany's winter oilseed rape cultivation area. In that year the hybrid cultivar 'Talent' replaced the once-popular line cultivar 'Express' as the most widely-cultivated winter oilseed rape variety in Germany, the first time a hybrid cultivar had achieved the top position. One of the most important reasons for the popularity of hybrid varieties is that they tend to have higher yield performance and stability than pure line, synthetic, or composite cultivars. A study on nitrogen acquisition and utilisation of oilseed rape has shown that hybrid cultivars showed better performance for both traits (Kessler 2000).

The increased yield potential of F1 hybrids in comparison to their parental inbred lines is known as heterosis. This phenomenon, the basis for breeding of hybrid cultivars, has been observed by numerous researchers in oilseed brassicas under a large range of test conditions (Schuster 1969, Grant and Beversdorf 1985, Lefort-Buson et al. 1987, Brandle and McVetty 1989, Friedt and Schilling 1991). For this reason, the exploitation of the heterosis effect in hybrid breeding of both winter and spring oilseed rape has become increasingly important in recent years. In rapeseed hybrids based on design trials, Zehr et al. (1997) recorded seed yield heterosis up to 27% compared to commercial varieties. In current winter oilseed rape material yield improvements of up to 15% have been reported for F1 hybrids compared to non-hybrid varieties.

One common method applied in hybrid breeding programs uses reciprocal recurrent selection (Comstock et al. 1949). Within this scheme it is important to determine 'heterotic pools', subpopulations that have a distant genetic background and supposedly show a high ability to express heterosis effects when crossed with members of other subpopulations. Certain mating designs, such as a diallel design (Griffing 1956) or a North Carolina design II (Comstock and Robinson 1948), can be applied to determine materials to be included in heterotic pools and to test crosses that may result in a high hybrid performance. However using such complex experimental designs for breeding is cumbersome and requires considerable resources. In some crop species, such as maize, it is relatively simple to generate such experimental crossing populations due to the technically simple castration of flowers from large numbers of individuals for production of pure F1 seed. This is not the case in oilseed rape, and only after the introduction of male-sterility technologies did it becomes feasible to develop F1 hybrid cultivars in this crop.

With the possibility to incorporate molecular markers into breeding programs, interest is increasing today in the potential use of DNA marker information to predict hybrid performance and hence reduce the time and cost of hybrid cultivar breeding. In oilseed rape, Lefort-Buson et al. (1987), Diers et al. (1996), Shen et al. (2003) and Qian (2007) tried to find a relationship between genetic distance and heterozygosity based on marker alleles, and to predict hybrid performance based on this relationship. These studies revealed that molecular marker-based genetic distance cannot always be used to predict hybrid seed yield and combining ability. One reason for this is probably the fact that hybrid vigour can only be accurately predicted on the basis of genetic distance if the molecular markers used for the distance estimation are linked to genes affecting the trait of interest (Charcosset et al. 1991, Bernardo 1996). One way of potentially identifying genes influenced by heterosis is to map molecular markers relevant to heterotic quantitative trait loci (QTL) in defined mapping populations, making use of QTL mapping techniques. Experiments aimed at mapping QTL involved in yield and yield-related traits in rapeseed have been conducted previously, in some cases involving hybrid materials (e.g. Quijada et al. 2006, Udall et al. 2006). However, reports on QTL that are directly relevant to heterosis in oilseed rape are still scarce.

1.1.1 Theoretical aspects of heterosis

Heterosis, the term that followed "heterozygosis" which was first used at the beginning of 20th century, was defined by Shull (1948) as "the increased size, the excessive kinetic energy, the increased productiveness, resistance to disease or to unfavorable conditions of the environment, the 'stimulating effects of hybridity' [.....] which may be observed in cross-bred organisms when compared with corresponding inbred or relatively more pure-bred organisms". In short, heterosis is "the increase in size or rate of growth of offspring over parents" (Duvick 1999). This is a phenotypic definition and heterosis is generally observed as a property of quantitative traits, hence the first theoretical explanation of heterosis was given through quantitative genetics.

Heterosis can be described in different ways: One formulation is the difference between the hybrid and the mean of the two parents, known as mid-parent heterosis. Falconer and Mackay (1996) explained the theoretical background of mid-parent heterosis based on the relationship between genetic distance (difference in allele frequency), a dominance effect and a heterosis effect, which had been observed by earlier workers. Later, Lamkey and Edwards (1998, 1999), based on a theoretical framework described by Willham and Pollak (1985), revisited and refined the theoretical relationship by differentiating heterosis at the population level and in an inbred line cross system. The former could be derived from the genetic architecture of both parents, whether they were from random-mating or inbred populations. These authors introduced the concepts of baseline heterosis, panmictic-midparent heterosis, and inbred-midparent heterosis. The inbred-midparent heterosis, which is the sum of baseline-heterosis and panmictic-midparent heterosis, is what has been generally exploited in the production of hybrid cultivars. Another particularly important point that emerged from the theoretical considerations of heterosis is that the performance of an F1 hybrid is a function of dominance and unlinked dominance interacting via dominance epistasis at loci showing genetic divergence.

The first hypothesis proposed as an explanation of heterosis was the theory of overdominance presented by East and Hayes (1912) and refined by East (1936). This idea assumed that "vigor is promoted when the genes at certain loci are unlike". On the other hand, Jones (1917) showed that heterosis "could result from normal gene action and be a phenomenon accompanying hybridity". This observation led to a second hypothesis

dubbed "dominance theory", although it can be better described as "avoidance of recessive deleterious genes" since the idea is based on heterozygous loci that prevent deleterious effects brought about by recessive genes. Rasmusson (1933) proposed a gene interaction hypothesis which was later called "epistasis theory". These three hypotheses still dominate the discourse on heterosis today, whereby different types of evidence can support or reject each of the different ideas. Numerous discussions of the three hypotheses, along with corresponding evidence reflecting current knowledge on this phenomenon, were reported at an International Symposium on the Genetics and Exploitation of Heterosis in Crops in 1997 (Coors and Pandey 1999).

Before the dawn of molecular biology and high-capacity computing devices, deciphering the basis of heterosis was based purely on quantitative genetics, mostly using linear models (Cockerham 1954). In a quantitative genetics sense, estimation of heterosis effects is actually a breakdown of genetic architecture into its components, dissecting phenotypic variation into additive genetic and dominance gene actions and their epistatic interactions (Hayman 1948, Mather and Jinks 1972, Griffing 1956, Comstock and Robinson 1948). Within a properly set mating design to develop certain types of generations, the heterosis effect is often treated as an effect of dominance, or the "specific combining ability" effect, especially when epistasis is ignored in the model.

Estimating heterosis effects using mating designs is not without problems. The models developed tend to ignore epistasis effects, since introducing epistasis requires considerably more materials. Designs involving partial mating to reduce the required resources were suggested (e.g. the partial diallel cross of Kempthorne and Curnow 1961), but these can still not completely account for epistasis. Another criticism is that analyses based on linear models tend to overweight the information from simpler effects, which explains why epistasis usually has no significant effect. Cheverud and Routman (1995) proposed that effects should be calculated without considering their frequency, so that epistatic effects could be better observed. With the emergence of high-capacity computers, calculation-intensive analysis methods such as *Best Linear Unbiased Prediction* (BLUP) using linear mixed models (Bernardo 1996) became suitable for such designs.

Thorough molecular genetic investigation opens new possibilities to elucidate the mechanism of heterosis, either by using markers as tools to determine genome regions

influencing heterosis at certain points in the chromosome, or by explaining heterosis at the molecular level (so-called molecular heterosis). Bernardo (1999) found that prediction of untested single crosses using molecular marker-assisted BLUP did not provide an advantage compared with phenotype-based BLUP, especially when many loci control the trait. Using evidence from human data, Comings and MacMurray (2000) proposed three explanations for single-locus molecular heterosis, which may not be mutually exclusive: optimal gene expression of the heterozygote, existence of independent contributing factors, and a broader range of expression of heterozygote genotypes. The proposal was supported by Song and Messing (2003) through comparison of relative expression of zein-expressing genes with their respective relative cDNA frequencies in maize grain; they find out that heterosis is likely not just an accumulation of dominant alleles, but rather the molecular phenotype of the heterozygous genotype itself determines the degree to which dominance influences heterosis. Because maximum levels of heterosis have obviously not yet been achieved in crop plants despite extensive breeding, Birchler et al. (2003) questioned the validity of allelic complementation to explain the dominance theory of heterosis. Evidence found in allopolyploid plants and studies on their inbreeding tended to contradict allelic complementation as a cause of dominant heterotic effects.

In the model system rice (*Oryza sativa*), Zhang et al. (2000) assessed the relationship between gene expression and heterosis by assaying differential gene expression in hybrids relative to their parents in a diallelic crossing scheme. Hybridisation of isolated cDNAs with RNA populations from seedling and flag leaf tissues detected an overall elevated level of gene expression in the hybrid compared with the parents. Detailed expression analysis revealed that differentially expressed cDNAs occurring in only one parent of the cross were positively correlated with heterosis. The genetic basis of quantitative traits and heterosis was generally found to be underlaid by large numbers of two-locus epistatic interactions. Huang et al. (2006) used microarray analysis to show that changes in biochemical and physiological activities were related to differential gene expression in the rice hybrid relative to the parents. Interestingly, they found that genes functioning in DNA replication and repairing tended to show positive heterosis, while genes functioning in carbohydrate, lipid, and energy metabolism, translation, protein degradation and cellular information processing showed negative heterosis. Genes

involved in amino acid metabolism, transcription, signal transduction, plant defense, and transportation were found to exhibit both positive and negative heterosis.

Although the quantity of data describing heterosis and its effects in crop plants has grown in recent years, very little knowledge exists to date regarding the contributions of the different potential effects on heterosis in rapeseed. The possible relationships among heterotic traits in different developmental stages (particularly seedlings) and their potential correlation to seed yield heterosis is completely unknown in oilseed rape.

1.1.2 Mapping of quantitative trait loci (QTL)

Mapping of quantitative trait loci, commonly known as QTL analysis (Lander and Botstein 1989), is today an important tool in plant breeding. Besides its direct practical application to support marker-assisted breeding, it provides vital information for studying the genetic architecture of complex traits (Holland 2007) and for localising parts of the genome involved in important traits as a first step in map-based cloning of relevant genes (Salvi and Tuberosa 2005).

The most common methods used in QTL mapping were developed during the late 1980s and early 1990s. Initially, Lander and Botstein (1989) invented interval mapping, which relates the intervals between pairs of linked markers to the phenotypic distribution of a trait. In this technique the estimation of QTL positions and effects uses the maximum-likelihood method. This is an extension from single-marker analysis, which relies on an association between a marker and the trait distribution based on common statistical methods, such as regression analysis or analysis of variance. An approximation of the interval mapping technique using least-square methodology was suggested by Haley and Knott (1992) and Martinez and Curnow (1992), respectively. This method gained popularity since it was fast and often gave similar results to the cumbersome maximum-likelihood technique. However, Kao (1995) warned that the least-square method should be treated as a preliminary test, which needs to be verified by maximum-likelihood based methods. Refinements of interval mapping suggested by Jansen (1993) and Zeng (1994) led to multiple interval mapping and composite interval mapping, respectively. These authors proposed the use of some markers as so-called “cofactors” for the interval being investigated, in order to uncover possible effects of markers that may not be detected

using interval mapping. The most recent QTL mapping methods integrate Bayesian models (Markov Chain Monte Carlo, MCMC) as the estimation method (Sillanpää and Arjas 1999) or even use a nonparametric concept (Zou et al. 2003).

The above-mentioned QTL mapping methods are usually based on clearly defined mapping populations derived from a biparental cross between two homozygous lines. Typical mapping populations used are F2-F3 families, doubled-haploid (DH), back cross (BC) populations or pools of recombinant inbred lines (RIL). The latter are particularly useful for QTL mapping since their genetic properties are clearly defined. In the case of QTL analysis in species that are impossible to self-fertilise, as is the case in animal breeding, information on kinship within the mapping population becomes extremely important.

In recent years a class of QTL mapping methods called *association mapping* (see e.g. Pritchard et al. 1996) was developed to deal with species for which the development of classical mapping populations is difficult or impossible (e.g. humans). Its application in the model plant *Arabidopsis* was promising (Thornsberry et al. 2001) and now it has been applied to at least eleven crops, including two tree species (Zhu et al. 2008) and oilseed rape (Hasan et al. 2008). Association mapping is based on the detection of linkage disequilibrium amongst markers and/or genes and their statistical associations with the phenotypic trait distribution. In plant breeding populations association mapping techniques offer a useful way to detect trait-related allelic diversity, for example in gene bank materials, since they are not restricted to the biallelic state that is intrinsic in a biparental cross. Moreover, these techniques are relatively cheap since the development of a defined mapping population is not a necessity.

1.1.3 QTL mapping of heterosis-relevant loci

Heterosis and the related phenomenon of inbreeding depression are related to fitness and are generally influenced by numerous genes and by environment (Mather & Jinks 1982). Most studies on the mapping of heterosis QTL in crop plants have attempted to dissect the genetic basis of heterosis and inbreeding depression in the model species rice. According to Xiao et al. (1995), heterosis in rice is mainly influenced by dominance complementation. Yu et al. (1997) reported overdominance at several major-effect QTL

with considerable additive-additive epistasis affecting grain yield and its components. Recent results in *Arabidopsis* also revealed the significant role of epistasis in heterosis. (Kusterer et al. 2007). Similarly, Li et al. (2001) and Luo et al. (2001) concluded that most QTL involved in heterosis and inbreeding depression in rice appeared to be involved in epistasis. Furthermore, 90% of the QTL they found to be involved in heterosis were overdominant. Seed yield and its component traits have been extensively studied in QTL mapping studies, particularly in cereals, and dissection of complex heterosis-relevant traits into component QTL can enable detection of a larger number of relevant loci. Particularly the contrasting results in rice (cf. Xiao et al. 1995, Yu et al. 1997, Li et al. 2001, Luo et al. 2001) demonstrate that the genetic control of heterosis can differ in different crosses. The possibility to investigate heterosis QTL in different oilseed rape populations would give useful insight into whether this also holds true for the much more complex, polyploid *B. napus* genome.

1.1.4 Synteny-based comparative mapping in *Brassica* using *Arabidopsis* resources

With an ever-growing resource of *Brassica* sequence data and its exact annotation to orthologous sequences in the *Arabidopsis* genome (see e.g. http://www.brassica.info and http://atidb.org/) it is today becoming increasingly possible – despite the complex rearrangements among *Brassica* genomes compared to *Arabidopsis* – to align and compare chromosomal and genomic data between the crop brassicas and the model species and to use this new information for genomic studies in the comparatively large genome of oilseed rape. Comparison between *Brassica* and *Arabidopsis* physical maps using published genome annotation and synteny data (e.g. Parkin et al. 2005) uncovers an enormous wealth of tools for fine-mapping, synteny-based gene cloning and marker development for marker-assisted selection. For example, online SSR search engines can be used to scan *Arabidopsis* or *B. rapa* chromosome regions flanking candidate genes of interest or major QTL positions (Hasan et al. 2008), and *Brassica* SSR primers that are identified in this manner can amplify polymorphic markers at one or more homologous loci in oilseed rape. Hasan et al. (2008) showed that linkage of such markers to QTL for a complex trait such as seed glucosinlate content could be confirmed by re-mapping to QTL regions or by allele-trait association analysis in genetically diverse genotypes. If such markers are in linkage disequilibrium with the gene of interest, this strategy can be extremely useful for indirect mapping of candidate genes on *Brassica* chromosomes.

Because SSR markers are codominant, this approach also has the potential to enable map localization of duplicated copies of a given candidate gene, for example to allow comparisons with major QTL positions.

1.2 Objectives

The overall aim of this project was a comparative analysis of the genetic control of heterosis in oilseed rape by QTL mapping of heterosis-relevant loci for biomass and yield traits at different developmental stages. A large population of 250 DH lines from a cross between two genetically diverse winter oilseed rape lines was used to create a genetic map for QTL analysis. Greenhouse and field trials of test hybrids from crosses between the individual DH lines with male-sterile tester lines were performed to enable the identification and dissection of QTL that correspond to the expression of heterosis in seedling biomass traits and seed yield, respectively.

Based on the results of the experiments the following to date unanswered questions will be addressed in this thesis:

a) Is it possible to identify quantitative trait loci that relate to heterosis for seedling biomass traits and seed yield in oilseed rape?

b) Are there common loci influencing heterosis for early biomass traits and seed yield?

c) How do these loci influence heterosis for the different traits (additive, dominance, overdominance or epistatic interactions)?

2 MATERIAL AND METHODS

2.1 Plant materials

The material used for this study comprises a doubled-haploid (DH) population of winter oilseed rape that was top-crossed with a common male-sterile parent to produce a back cross (BC) population. The production of the materials involved two steps: First, a DH population was generated from a cross between an inbred line ('Express 617') of the German winter oilseed rape cultivar 'Express' (00-quality: low seed glucosinolate, zero erucic acid content) and the genetically diverse semi-synthetic breeding line 'V8' (++ quality: moderate glucosinolate, high erucic acid content). A total of 250 DH lines were produced via microspore culture from a single F1 plant of this cross (Spiller 2006). Seed increase of the DH lines was achieved by self-pollination in isolation tents using solitary bees (*Osmia cornuta*) as pollinators. In the second step, a population of 250 corresponding test-hybrids were produced from the DH lines by controlled crossing with the common female parent 'MSL-Express', a male-sterile line that is derived from the parental genotype 'Express 617'. The genetic architecture of the population of the test hybrids is the same as a BC population, since it contains two possible genotypes for any given locus, namely homozygous for 'Express 617' alleles, or heterozygous with one 'Express 617' and one 'V8' allele, respectively. The expected segregation ratio between homozygous and heterozygous individuals at any given locus is 1:1. Because the test hybrids only have a maximum of two alleles at each locus, it is theoretically possible to estimate additive and dominance effects of QTL contributing to heterosis.

2.2 Genotyping and genetic mapping

2.2.1 DNA extraction

Young leaf material from three-week old plants was collected from each of the 250 DH lines of the mapping population plus the two parental lines 'Express 617' and 'V8'. Genomic DNA was extracted following the method described by Doyle and Doyle (1990). The DNA samples were stored as aliquots at -20°C until they were used for marker screening.

2.2.2 Genotyping

A total of 256 AFLP® primer combinations, derived from combinations of the restriction enzymes *Eco*RI and *Mse*I, were screened in the cross parents to determine the most polymorphic primer combinations for the subsequent genetic analysis. The 77 primer combinations that showed the highest levels of polymorphism between the cross parents were applied on the 250 DH lines. AFLP® analysis was performed using the standard procedure described by Vos et al. (1995). For unambiguous identification of linkage groups, a total of 797 simple sequence repeat (SSR) markers were also screened for polymorphism between the parents. Most of the SSR primers used are publicly available (Suwabe et al. 2002, Lowe et al. 2004, Piquemal et al. 2005); 96 were commercial SSR primer combinations kindly provided by the Saaten Union Resistenzlabor, Leopoldshöhe, Germany. The protocol used for SSR marker genotyping follows the usual PCR procedures using Mg^{2+} salt as cofactor for the *Taq*-polymerase. The optimal annealing temperature was used whenever known; otherwise, a "touch-down" PCR scheme was applied as described by Hasan et al. (2008). LICOR® polyacrylamide gel electrophoresis with 48 lanes was applied to score marker bands for each AFLP and SSR primer combination in the mapping population and parental genotypes.

2.2.3 Genetic mapping

A total of 527 polymorphic AFLP® markers and 176 SSR markers were used to construct a genetic map using the software JoinMap® 3.0 (Kyazma, B. V., Wageningen, The Netherlands; see Van Ooijen and Voorrips 2001) with Kosambi's mapping function and a maximum likelihood distance of 40 cM. Prior to QTL analysis, cosegregating markers and markers with genetic distance lower than one centimorgan (cM) were removed from the map, whereby preference was given to SSR markers when these cosegregated with AFLP® markers. The result was a map covering 1792 cM, with 19 linkage groups containing 269 markers, 144 of which are SSR markers.

2.3 Field trials

The mapping population of 250 DH lines and the corresponding population of 250 BC test hybrids from the DH lines were evaluated at four locations in Germany during the growing

seasons 2005/06 (sowing at September 2005 and harvest in August 2006) and 2006/07. The locations used were Rauischholzhausen and Grund-Schwalheim (in cooperation with SW Seed GmbH) in Middle Hesse, along with Reinshof (in cooperation with the University of Göttingen) and Einbeck (in cooperation with KWS Saat GmbH) in Lower Saxony.

The field trials at each location were performed using an alpha-lattice design (Patterson and William 1976) with 26 blocks of 26 plots each. Randomisation was conducted using ALPHA+© ver. 2.4 (1998) from CSIRO and Biomathematics and Statistics Scotland. Due to the very large size of the trial at each location it was necessary to ensure that each DH line was grown in a position close to its corresponding test hybrid, in order to exclude performance differences between the lines and their hybrids that were due to positional effects rather than heterosis. For this reason each block was divided into two halves that were sown parallel to each other. One half contained 10 plots of DH line; while the opposite half of the block contained the back cross hybrids corresponded to these DH lines. Due to the large size of the trial, it was not feasible to include two replications per location, except for the standards, which had five replicates. Consequently, locations became replicates in the analysis and genotype-location interaction effects could not be estimated.

Estimates of yield potential were calculated from the harvest of each plot, after conversion to dt per ha (10^{-1} metric ton.ha^{-1}) with a seed water content of 91%. Besides the seed yield, thousand seed mass (TSM) was calculated as a measure of seed size. Plant height was observed at the end of flowering.

2.4 Greenhouse trial

A greenhouse trial was conducted in the IFZ Research Centre greenhouse facilities at Justus Liebig University, Giessen, to study heterosis during seedling development. The greenhouse trial used the same populations of DH lines and their test hybrids that were used in the field trial, in order to enable direct comparisons between heterosis for seedling traits and for yield, and their respective QTL.

Biomass accumulation was approximated through fresh and dry mass at 28 days after sowing (das). The seedling growth was estimated by measuring cotyledon height at 14 das. Leaf area was measured at 28 das as an approximation of the functional assimilation area, and also served for estimation on specific leaf weight (SLW, dry leaf biomass per unit leaf

area). SLW, as well as its reverse counterpart, the SLA, is often used to measure the "leafiness" of a plant; it indicates the "thickness" of the leaf per unit area and thus gives an indirect indication of photosynthetic characteristics of a leaf, while giving a direct measurement of allocations of biomass in a plant (Jurik 1986).

Seedlings of each accession were grown in the greenhouse in nine pots of size 10 cm × 10 cm. Initially three seeds per pot were sown into soil as growth medium, and each pot was thinned to one seedling per pot at seven days after sowing (das). The nine pots of each genotype were arranged in a 3 × 3 pattern, and the lines and their respective hybrids were always positioned adjacently to ensure that each line and its corresponding test hybrid were grown under the same temperature, water and light conditions. An alpha-lattice design with two replicates was applied as a randomisation scheme, using twenty blocks comprising 13 pairs of accessions that were each sown on the same day.

After seven days the seedlings that were thinned from each pot were used for the first observations of total shoot fresh weight (7 das). At 14 das, the timepoint when the epicotyls generally started to appear, cotyledon height was measured on five plants per genotype without removing the seedlings. The shoot fresh weight of the nine remaining plants of each genotype was measured at 28 das by weighing directly after harvesting. Finally, two plants per genotype were sampled for measuring the leaf area. The leaves were scanned using a HewlettPackard Scanjet 5400c flatbed scanner and the images were processed using ImageJ v. 1.37 (http://rsbweb.nih.gov/ij/index.html) to calculate the leaf area.

2.5 Statistical analysis

PROC MIXED from SAS® 8.02 was employed to analyse the observation data and to estimate least square means (LS means) of the trait values for each accession. The analysis of data from the field trials was conducted for each year individually as well as pooled over years. The `model` statement was arranged following Piepho et al. (2003), based on the trial design applied. Accession, location (as replicate), year and accession-to-year interaction effects were considered random.

2.6 Quantitative trait loci (QTL) analysis

A quantitative trait analysis was applied to the greenhouse and field trial data to obtain information on the positions and effects of QTL in the 'Express 617' × 'V8' genetic map. The QTL analysis was conducted using the freely available software QTL Network 2.0 (Yang et al. 2005). The method used was composite interval mapping (Zeng 1994). Threshold levels were set to the equivalent of 0.005 value of probability for QTL detection to be declared as significant. For each trait, three sets of data based on the different population datasets were analysed: DH population, BC population and midparent heterosis data (MPH). For the QTL analysis all of the three sets of data were analysed using the `_Population DH` option. As a result, when interpreting the genetic effects measured by the software for each dataset, the genetic architecture of the respective population needs to be taken into account.

2.7 Interpretation of genetic effects

2.7.1 Single locus case

The option `_Population DH` in QTL Network 2.0 will pool data into two groups: Those whose genotype code is the same as first parent (P1) and those who have the same code as the second parent (P2), with the corresponding means $\overline{P1}$ and $\overline{P2}$, respectively. The calculated additive genetic effect estimate *a* is provided both by QTL Mapper (Wang et al. 1999) and by QTL Network 2.0 (Yang et al. 2005) using the simple formula

$$\underline{a} = (\overline{P1} - \overline{P2})/2 .$$

Since in the analysis all types of dataset/population (DH, BC, and MPH) were treated in the same way as DH data, this calculation leads to different genetic interpretations depending on the population, as follows:

DH line dataset

Assuming A_1A_1 is the genotype from parent 1 (P1, 'Express 617') at locus A, and A_2A_2 is the genotype from parent 2 (P2, 'V8'). The population of DH lines comprises a mixture of two homozygous genotypes for this locus: A_1A_1 and A_2A_2 at an expected ratio of 1:1.

Using Fisher's notation, the genotypic value of A_1A_1 is a, whereas A_2A_2 has the value $-a$ (Falconer and Mackay 1997). Thus, in this population, $\underline{a}$ estimates the additive genetic effect a in the following way:

$$\underline{a} = (\overline{P1} - \overline{P2})/2 = \hat{a}\ .$$

Where $\overline{P1}$ and $\overline{P2}$ are average values of individuals with genotypes A_1A_1 and A_2A_2, respectively, at locus A. If $\overline{P1} > \overline{P2}$, the value is positive.

BC dataset

The BC hybrid population comprises, for any given locus, a mixture of homozygotes with two copies of the allele (e.g. A_1) from parent 1 ('Express 617') and heterozygotes with one allele from each parent (A_1 A_2): The expected segregation for A_1A_1 and A_1A_2 is 1:1. In the scoring of the genotypes for mapping, P1 pools individuals with genotype A_1A_1 (coded as 'a' in the marker scoring), whereas P2 pools individuals with A_1A_2 genotypes (coded as 'b'). Following Fisher's notation, the genotypic value of the latter is written as d. In this case, $\underline{a}$ estimates

$$\underline{a} = (\overline{P1} - \overline{P2})/2 = (a - \overline{d})/2$$

or half the difference between the additive genetic and dominance effects. The interpretation of the value needs information from the value of parent 2 ('V8') because d is calculated as the deviation from the midparent value.

Midparent heterosis dataset

The midparent heterosis (MP) dataset is calculated for each pair of lines and hybrids using the formula

$$MP_i = BC_i - (DH_i + 'Express')/2$$

where index i denotes the DH line number (1 to 250). Again the MP data are divided into two groups: The first, P1, pools samples with the code 'a' and the mean value $\overline{P1}$, while the second, P2, pools samples with the code 'b' and the mean value $\overline{P2}$. The $\overline{P1}$ is expected to have the value of 0, since the BC and DH parents have the same genotype as the recurrent parent 'Express 617', A_1A_1. Meanwhile, $\overline{P2}$ is expected to have the same

Table 2.1 Genetic interpretation of genotype from a single QTL.

Dataset	Direction of positive contribution[1]	Situation	Genetic interpretation of $\underline{a}$
DH	'Express'	$\overline{P1} > \overline{P2}$	*a*
	'V8'	$\overline{P1} < \overline{P2}$	*-a*
BC	'Express' and 'V8'	$\overline{P1} > \overline{P2}$	*(a-d)/2*
	'Express' and 'V8'	$\overline{P1} < \overline{P2}$	*-(a-d)/2*
	'Express'	$\overline{P1} > \overline{P2}$	*(a+d)/2*
	'V8'	$\overline{P1} < \overline{P2}$	*-(a+d)/2*
	none	$\overline{P1} > \overline{P2}$	*-(a-d)/2*
	none	$\overline{P1} < \overline{P2}$	*(a-d)/2*
MPH	'Express'	$\overline{P1} > \overline{P2}$	*-d/2*
	'V8'	$\overline{P1} < \overline{P2}$	*d/2*

[1] Positive contribution as taken when mid-parent value is set to zero.

value as *d*, since the BC individuals have the genotype A_1A_2 and the DH lines have the genotype A_2A_2. Thus,

$$\underline{a} = (\overline{P1} - \overline{P2})/2 = (0 - \hat{d})/2 = -\hat{d}/2$$

Or $\hat{d} = -2\underline{a}$. A positive $\underline{a}$ value (when $\overline{P1} > \overline{P2}$) is half the negative value of *d*.

2.7.2 The case of two-locus epistasis

As stated in the QTLMapper 1.0 Manual (Wang et al. 1999, p. 37):

> "A positive $\mathbf{AA_{ij}}$ value implies that the two-locus genotypes being the same as those in P1 parent or P2 parent take the positive effects, while the two-locus genotypes of recombination between the P1 parent and P2 parent take the negative effects. The case of negative $\mathbf{AA_{ij}}$ values is just the opposite."

This implies that the parental (P1 and P2) genotypes are the reference for the given sign of the effect. The recombinant genotypes will take the opposite sign. This is the result of the

product of coefficients of contrast attributed to additive effects for both loci in the design matrix for the interaction columns.

Recall that P11 and P22 are the groups of data with *parental* types and their average values are $\overline{P11}$ and $\overline{P22}$, respectively. The *recombinant* types in this case are P12 and P21, and their average values are $\overline{P12}$ and $\overline{P21}$, respectively. Due to the interactive nature of epistasis, the additive-to-additive epistasis effect $\underline{aa}$ is estimated using the following simple formula

$$\underline{aa} = (\overline{P11} + \overline{P22} - \overline{P12} - \overline{P12})/4 .$$

DH line dataset

Suppose there are two loci, A and B, whose alleles in the two parents are denoted A_1 and A_2 and B_1 and B_2, respectively. In a DH population, the group P11 pools individuals with the $A_1A_1B_1B_1$ genotype (a–a at both loci), while the group P22 pools the individuals with the $A_2A_2B_2B_2$ genotype (b–b). For the recombinant types, P12 and P21 pool the genotypes $A_1A_1B_2B_2$ (a–b) and $A_2A_2B_1B_1$ (b–a), respectively. Because

$$\overline{P11} = \mu + a_A + a_B + aa_{AB} ,$$
$$\overline{P12} = \mu + a_A - a_B - aa_{AB} ,$$
$$\overline{P21} = \mu - a_A + a_B - aa_{AB} \text{ and}$$
$$\overline{P22} = \mu - a_A - a_B + aa_{AB} ,$$

we have, for the DH line dataset, the following estimate for two-locus epistasis:

$$\underline{aa} = (\overline{P11} + \overline{P22} - \overline{P12} - \overline{P21})/4 = \widehat{aa_{AB}}$$

where aa_{AB} is the additive-to-additive genetic interaction effect for the respective pair of loci. The other components cancel each other out. Notice that the positive sign of the estimates of aa_{AB} means that the epistasis favours the parental genotypes, and the negative sign favours recombinant genotypes. It should be remembered that this epistatic component is estimated from the dataset from which the heterosis arises.

BC dataset

In data from a BC population, the group P11 pools individuals with the $A_1A_1B_1B_1$ genotype (a–a at both QTL) and the group P22 pools individuals with the $A_1A_2B_1B_2$ genotype (b–b). For the recombinant types, P12 and P21 pool the genotypes $A_1A_1B_1B_2$ (a–b) and $A_1A_2B_1B_1$ (b–a), respectively. While $\overline{P11}$ has the same definition as mentioned before, the other pools now have new interpretations:

$$\overline{P12} = \mu + a_A + d_B + ad_{AB},$$
$$\overline{P21} = \mu + d_A + a_B + ad_{BA} \text{ and}$$
$$\overline{P22} = \mu + d_A + d_B + dd_{AB}.$$

This will imply that the estimate takes the form of

$$\underline{aa} = (\overline{P11} + \overline{P22} - \overline{P12} - \overline{P21})/4 = (aa_{AB} - ad_{AB} \widehat{-ad}_{BA} + dd_{AB})/4$$

The interpretation of the expression is somewhat complex as it depends on the direction and size of the effects relative to the midparent values at both loci.

MP dataset

The MP dataset provides another genetic interpretation. The group P11 pools the samples with a–a genotype codes in both loci. The group P22 pools samples with the genotype code b–b, with an expected value of $d_A + d_B + dd_{AB} - aa_{AB}$. The group P12 pools samples with the genotype code a–b and an expected value of $d_B + ad_{AB}$, while the group P21 pools samples with the genotype code b–a and an expected value of $d_A + ad_{BA}$. Estimated values of each group are

$$\overline{P11} = \mu + a_A + a_B + aa_{AB} - (\mu + \mu + a_A + a_B + aa_{AB} + \mu + a_A + a_B + aa_{AB})/2 = 0$$
$$\overline{P12} = d_B + ad_{AB},$$
$$\overline{P21} = d_A + ad_{BA}, \text{ and}$$
$$\overline{P22} = d_A + d_B + dd_{AB} - aa_{AB}$$

Using these genetic interpretations we can calculate the two-locus epistasis as

$$\underline{aa} = (\overline{P11} + \overline{P22} - \overline{P12} - \overline{P21})/4 = (dd_{AB} - ad_{AB} \widehat{-ad}_{BA} - aa_{AB})/4$$

A positive value of $\underline{aa}$ indicates a greater contribution from dominance-to-dominance interactions, in the form of its difference from additive-to-additive interactions. If $\underline{aa}$ is negative, the main source of epistasis comes from additive-to-dominance interactions. It is worth noting that by subtracting the $\underline{aa}$ value obtained from the BC dataset from the $\underline{aa}$ value from the MP dataset, we have an estimate of the additive-to-additive interaction (epistasis) that is involved in heterosis. This means that we may obtain two estimates of aa_{AB} whenever QTL from the three sets of data occur in the same region/locus. However, estimates obtained from the subtractions are expected to have greater variances.

3 RESULTS

3.1 Genetic map

From 797 publicly available SSR primer pairs, 422 were tested in the two parents. Of these, 230 pairs did not amplify, 119 pairs amplified monomorphic fragments, and 73 were polymorphic. Additionally, 32 (from 35) SSR primer pairs provided by Saaten Union Resistenzlabor GmbH and 42 (from 114) primer pairs from the Celera AgGen Brassica Consortium (see Piquemal et al. 2005) were found to be polymorphic. The polymorphic primer pairs were used to genotype 262 DH lines.

The mapping enabled integration of 703 markers into 22 linkage groups, which were then reduced to 19 linkage groups since three groups were too small (only two or three markers). After removing redundant markers, the total map covered 1787.3 cM in 19 linkage groups that could be assigned to *B. napus* chromosomes N01 to N19 (Appendix A). With 419 markers mapped, the average marker-to-marker distance is 4.5cM. For QTL mapping purposes, markers with a distance smaller than 1cM were discarded, resulting in a backbone map containing 269 markers with an average distance of 7.2cM between markers.

3.2 Phenotypic observations: Greenhouse experiment

3.2.1 Shoot fresh and dry weight

As expected, shoot fresh and dry weight showed a strong correlation in all the datasets studied: DH (r = 0.88), BC (r = 0.83), and MPH (r = 0.80). Hence it can be concluded that the proportion of water content from all materials was relatively at the same level.

The distributions of shoot fresh weight and dry weight of both the DH and BC populations were skewed slightly to the right in the BC population (Fig. 3.1 and 3.2). The DH mean values were 12.15 g and 0.72 g for shoot fresh weight and dry weight, respectively, whereas the corresponding mean values in the BC population were 13.26 g and 0.77 g, respectively. Further investigation on correlations of both traits between the

DH and BC populations showed that the DH performance has only a weak, albeit significant, effect on its BC counterpart (Fig. 3.3). This indicated a slight contribution of covariance between the DH and BC populations.

The level of MPH calculated ranged from -24% (DH 571) to 43% (DH 199) for fresh weight and -30% (DH 114) to 84% (DH 199) for dry weight.

3.2.2 Leaf area

The leaf area distribution of the BC and DH populations showed the same pattern as shoot weight. A slight shift of means was also detected (Fig. 3.4). The mean values were 549.6 cm^2 and 579.7 cm^2 for the DH and BC populations, respectively. The correlation coefficient between the two populations was 0.27 ($P < 0.001$; Fig. 3.5), again indicating slight covariance between the DH and BC populations. The similar distribution pattern of leaf area to that for shoot weight was confirmed by a correlation coefficient for shoot fresh weight of 0.68 for the DH population and 0.66 for the BC population. Calculation of MPH for leaf area revealed a range from -31% (DH/BC pair #33) to 45% (#345). Among all the accessions, 89 (36%) pairs of accessions had a negative MPH value.

3.2.3 Hypocotyl length

Hypocotyl length was observed at 14 das and used as an approximation of seedling growth rate. Comparison of the hypocotyl length data distribution from the DH lines with the BC population showed, again, that the BC distribution shifted slightly to the right (Fig. 3.5). A unique feature, relative to the other traits measured, was that both distributions tended to skew to the right with a weak second peak. The correlation between the DH lines and the BC population indicated a strong association between the two datasets ($r = 0.81$, $P < 0.001$). The range of the MPH distribution for hypocotyl length (-14.6% to 54.1%) showed that most of the accessions had positive values. Only 14 accessions had BC values lower than their respective midparent value.

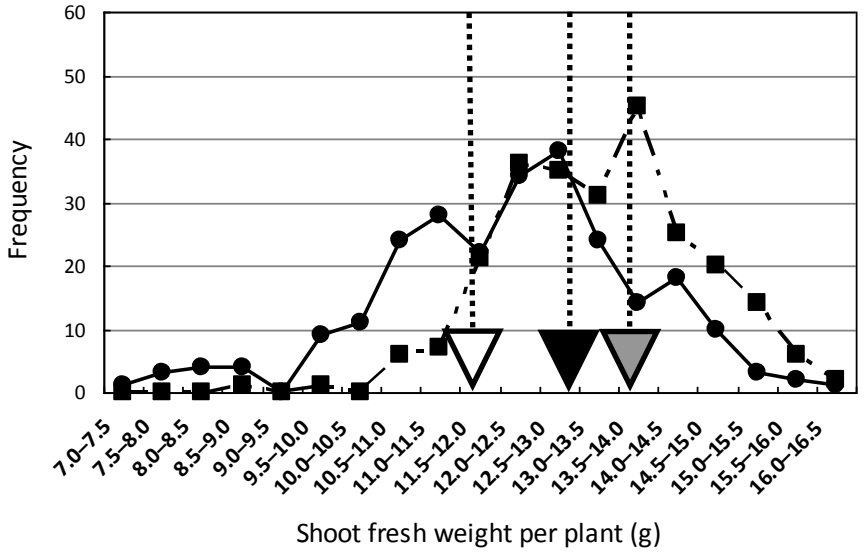


Figure 3.1 Shoot fresh weight distribution of 250 DH lines (straight line) from the DH population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express' (broken line). The triangles show means of parents: 'Express 617' (black) and 'V8' (white), as well as their F1 (grey).

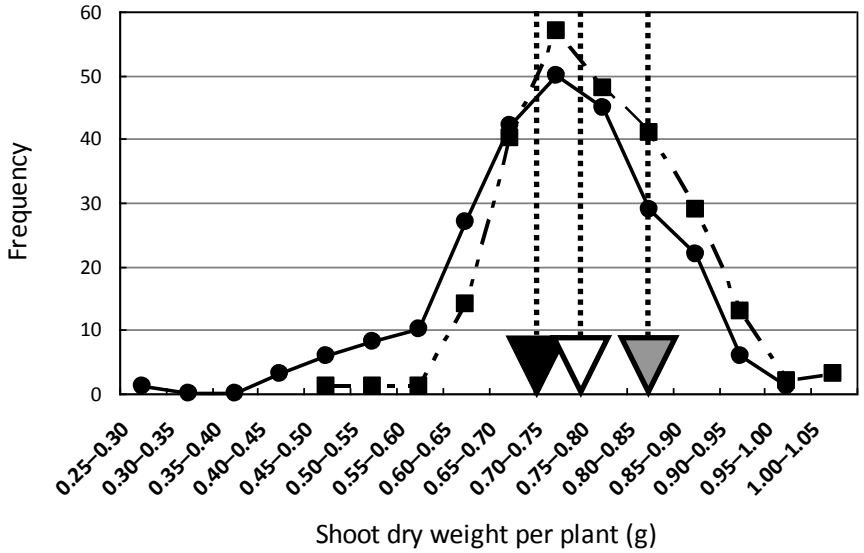


Figure 3.2 Shoot dry weight distribution of 250 DH lines (straight line) from the DH population 'Express 617' × 'V8' (white bars) and their respective BC test hybrids with 'MSL-Express' (broken line). the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey).

(a)

(b)

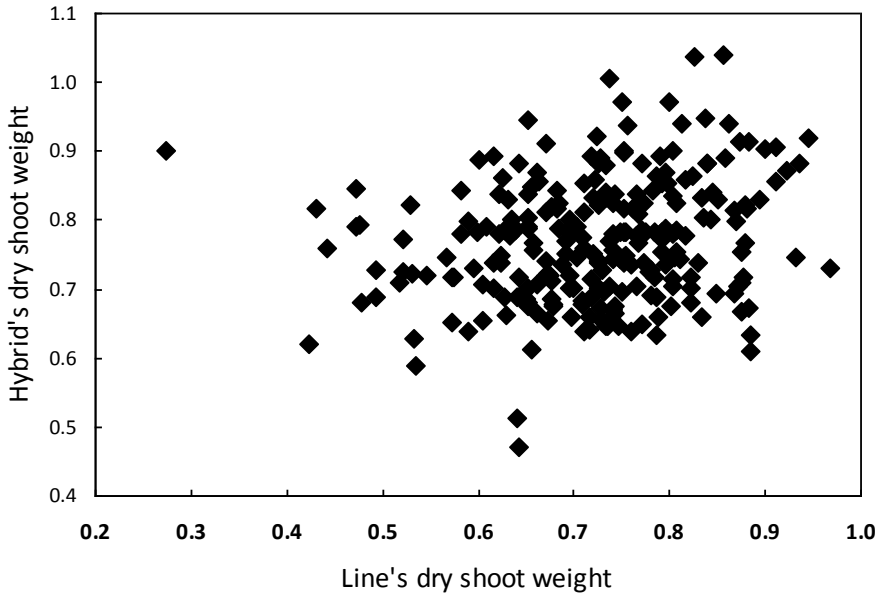


Figure 3.3 Relationship of (a) fresh and (b) dry shoot weight from the DH population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express' (grey bars). The correlation coefficients were 0.34 ($P < 0.001$) for fresh weight and 0.20 ($P = 0.001$) for dry weight, respectively.

3.2.4 Specific leaf weight

The specific leaf weight (SLW), calculated as the ratio of leaf dry weight to leaf area, was taken as an indicator of dry mass accumulation per leaf unit area. This trait is usually regarded as a measure of the capacity of a leaf to store biomass. Comparison of the SLW data distributions from the DH lines and the BC population showed that the DH lines had a broader variation than their BC progenies, with a lower frequency on the modal interval (Figure 3.6). The MPH values for SLW ranged from -29% for #114 and #345 to 49% for #199. From all DH/BC combinations tested, 104 (42%) pairs had negative MPH.

3.3 Phenotypic observations: Field trials

The field yields in the two growing seasons (2005/06 and 2006/07) showed a clear difference. The 2006/07 growing season was characterised by unusually mild winter temperatures that led to earlier flowering, followed by a dry period during the peak flowering season in April. The mean temperature in January and February 2007 was more than 3°C higher than average. In 2007 the normally wet month of April, when monthly precipitation of more than 30 $lt.m^{-2}$ can be expected, experienced 0.0 $lt.m^{-2}$ in both locations in Hesse (Rauischholzhausen: RH; Grund-Schwalheim: GS) and 3.2 $lt.m^{-2}$ in southern Lower Saxony (Reinshof, Göttingen: RE). This resulted in significantly lower yields in the 2007 harvest compared to 2006: on average 30% lower in GS, 23% in RE, and 36% in RH (Fig. 3.7). The fact that the differences in yield were location specific was supported by a statistically significant genotype-year interaction ($P = 0.0011$). Based on this, the estimation of yield, thousand seed mass (TSM) and plant height was performed individually for each planting year and not pooled.

3.3.1 Yield

Yields of the DH lines had a stronger correlation between the two harvest years in comparison to the BC and MPH datasets (Fig. 3.8). The DH population yields were also distributed over a wider range with lower mean, relative to the BC population. This indicated that the performance of the DH lines tended to be more stable to environmental (year) influence, whereas the BC population was more affected by environment but overall had a more uniform performance. The pattern of the MPH dataset resembled that

of the BC. Unexpectedly, the correlation coefficient of field yield between the populations for the 2005/06 and 2006/07 harvest gave similar patterns. The correlations between DH lines and BC were moderate and positive (0.47 for both harvest years), whereas between DH lines and MPH they were negative (-0.27 and -0.46 for 2005/06 and 2006/07, respectively). Although also moderate, the BC population had slightly higher correlations with MP than did the DH. This result may be influenced by the calculation used to generate the MPH data; however, the possibility that the BC population exhibited a stronger effect on yield heterosis than the DH lines was not unexpected.

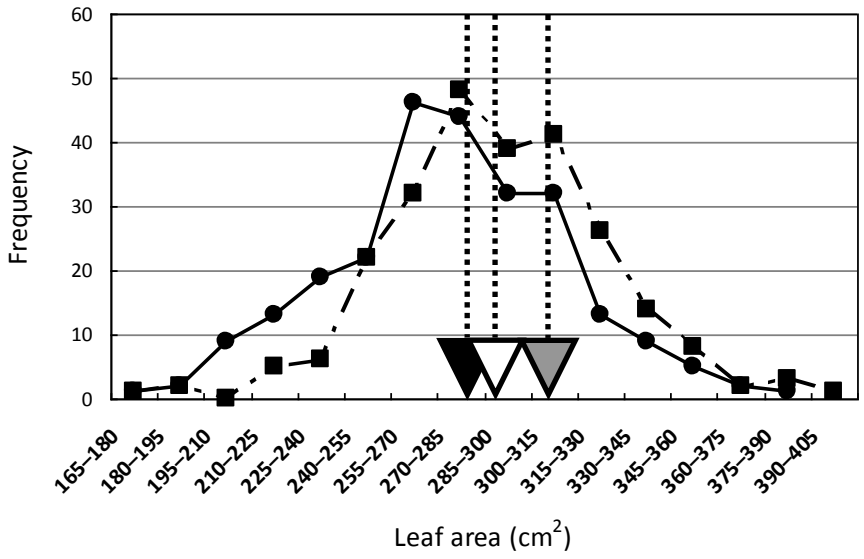


Figure 3.4 Leaf area distributions ‘for 250 DH lines from the DH population ‘Express 617’ × ‘V8’ (white bars) and their respective BC test hybrids with ‘MSL-Express’ (grey bars). Triangles show the trait values of the cross parents ‘Express 617’ (black) and ‘V8’ (white) and their F1 hybrid (grey).

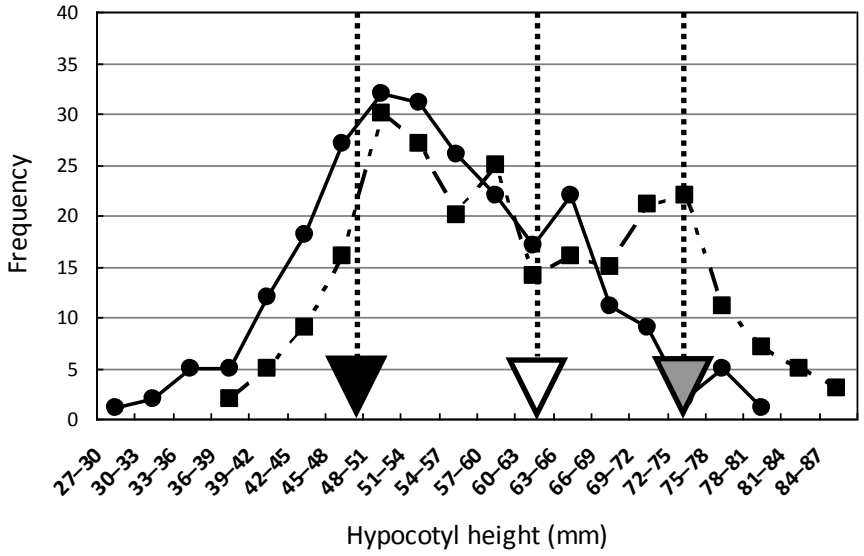


Figure 3.5 Hypocotyl length distributions of 250 DH lines from the population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. Triangles show the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey).

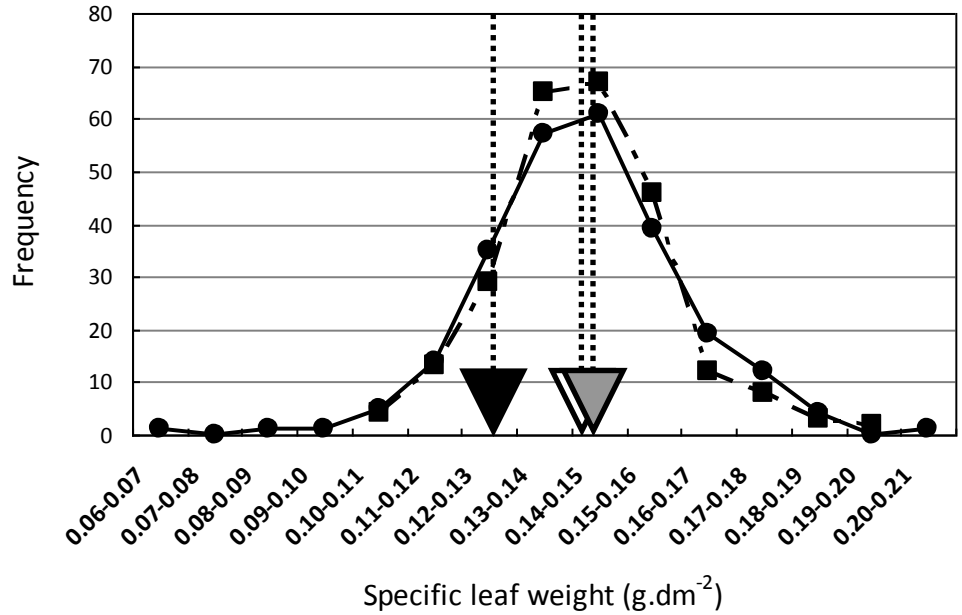


Figure 3.6 Specific leaf weight ($g.dm^{-2}$) distribution of 250 DH lines from the population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. Triangles show the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey).

The frequency distributions of yield data from 2005/06 and 2006/07 harvests showed that the range for the DH population was broader than for the BC population, and the modus of the BC population located at a higher yield value than the DH population (Fig. 3.9).

The absolute mid-parent heterosis (MPH) values from the 2005/06 harvest ranged from -7.25 dt/ha (#429) to 6.70 dt/ha (#213). From all accession pairs, 61 had negative MPH for plot yield in 2005/06. In contrast, MPH values from the 2006/07 harvest ranged from -3.8 dt/ha (-10.9%, #208) to 8.6 dt/ha (29.3%, #18) and only 11 pairs of accessions had a negative value (Figure 3.8). The MPH for the 2006/07 harvest turned out to have more positive values than 2005/06, although nominally they were lower. The drop in yield of the parent 'Express 617' to only 35% of the yield attained in the previous year (greater than the average yield reduction in the progenies) was detected and might be attributed to the sub-optimal situation of 2006/07 planting year.

3.3.2 Thousand seed mass (TSM)

Thousand seed mass had no significant relationship with yield, with correlation coefficients for the DH, BC and MPH populations being invariably near zero. None of the correlations were significant ($P \geq 0.001$). A strong correlation was observed between the TSM of the DH lines harvested in 2005/06 and in 2006/07 ($r = 0.84$, $P < 0.001$), reflecting the high stability of this trait in oilseed rape. The BC showed a more moderate correlation ($r = 0.41$, $P < 0.001$), whereas the MPH data showed the weakest correlation ($r = 0.25$, $P < 0.001$). This resembled the pattern of correlations seen for plot yield in each datasets. As shown by the scatter diagrams in Fig. 3.10, the DH lines tended to spread over a wider range for TSM relative to the BC population and the MPH data.

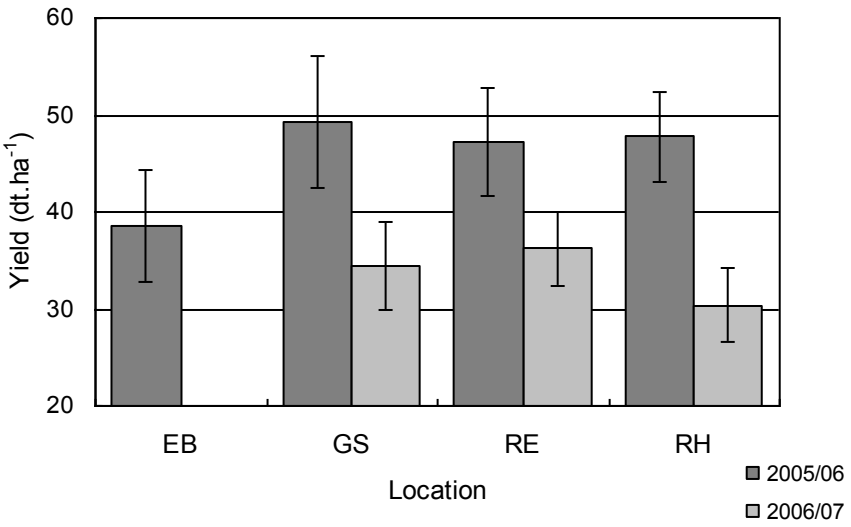
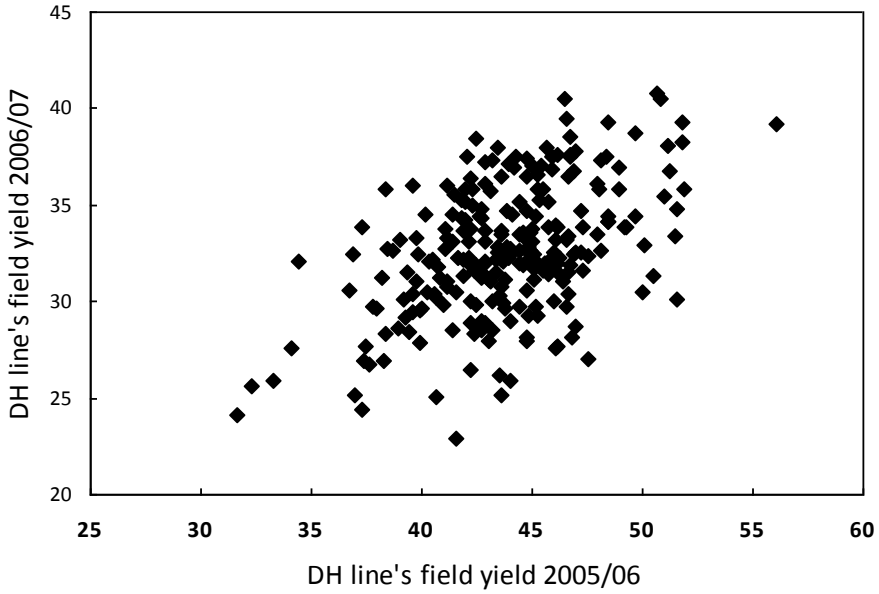
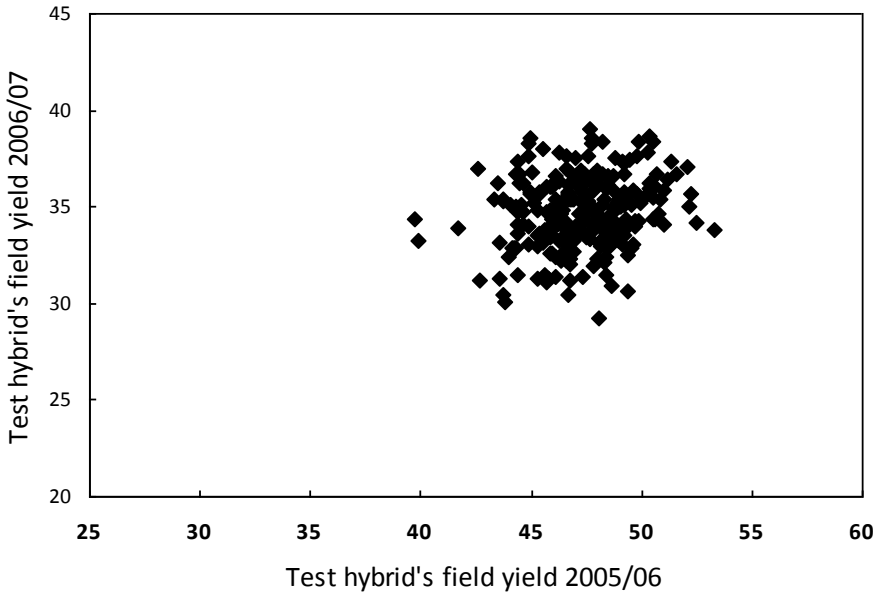


Figure 3.7 Means (arithmetic) of overall yield from the four trial locations Einbeck (EB), Grund-Schwalheim (GS), Reinshof (RE) and Rauischholzhausen (RH), in 2005/06 (darker) and 2006/07 planting year. No data was available from Einbeck in 2006/07 due to hail damage. Error bars show standard deviation ranges.

(a)



(b)



(c)

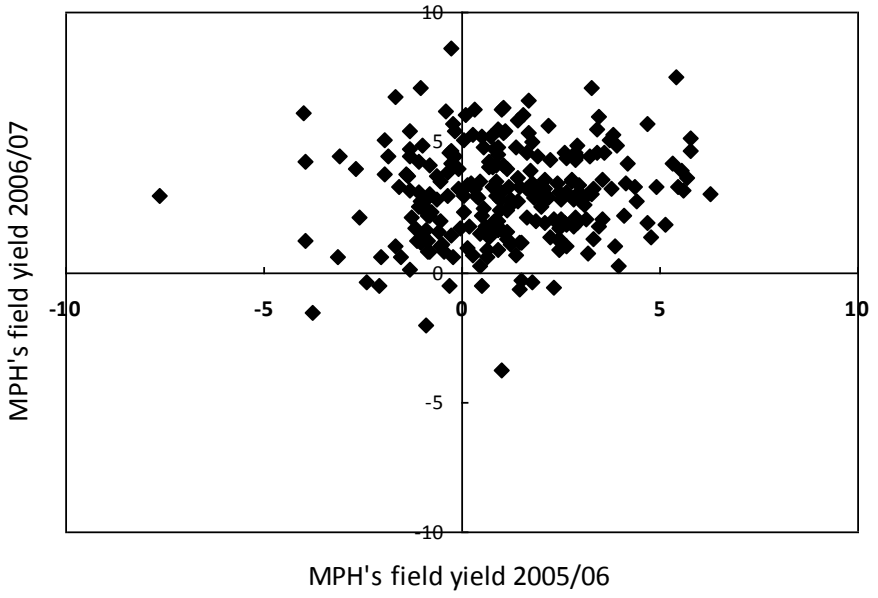


Figure 3.8 Scatter diagrams showing the relationships between seed yield in 2005/06 and 2006/07 for (a) 'Express 617' × 'V8' DH lines, (b) their BC test hybrids with 'MSL Express', and (c) mid-parent heterosis (MPH) of the BC test hybrids. Diagrams for the DH and BC populations are made with the same scale to emphasise their relative distributions.

Between populations, the correlation coefficients for TSM were moderate to high. The DH and BC populations showed a relatively high correlation (0.77) for 2005/06 and a moderate one (0.56) for 2006/07, whereas the DH population was moderately negatively correlated to the MPH data (-0.57 and -0.44 in 2005/06 and 2006/07, respectively). The correlations between the BC and MPH data were very different in the two harvest years (0.09 and 0.50, respectively), indicating that for TSM the change in heterosis performance was controlled more by a change in the performance of the hybrids. The effect of the respective DH lines was relatively stable.

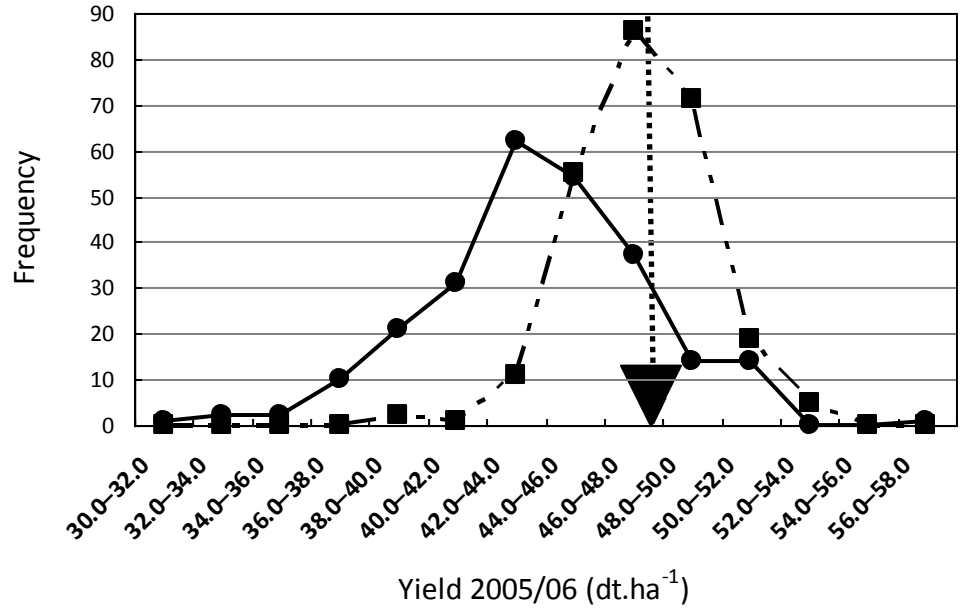
Frequency distributions of the TSM data for both harvest years are presented in Fig. 3.11. Again it is seen that the DH population was distributed more broadly than the BC population for both years, indicating a tendency of BC toward uniformity, whereas the high correlation between the DH data from 2005/06 and 2006/07 indicated an inert situation. Unlike the yield, which showed a reduction in the 2000/07 harvest, TSM did not show a dramatic change. The distributions showed quite similar patterns to yield patterns, although the BC population distribution tended to have the modus at a higher value of TSM. However, the ranks of the BC accessions in the two harvest years were dissimilar.

The MPH for TSM was weak, since the hybrid values were clustered around their respective mid-parent values. In 2005/06, 95 accession pairs had negative MPH values for TSM, with a range from -0.49 (#17) to 0.43 (#95). In 2006/07, 171 of the 250 accession pairs had negative MPH values, ranging from -0.92 (#17) to 0.53 (#165). DH 17 was the best performing DH line, and because its BC performed only moderately the MPH value for this pair was considerably reduced.

3.3.3. Plant height

The same pattern of correlations seen for the two other traits was observed for plant height, measured at the end of flowering. The DH population showed a stronger correlation between the years than the other two sets of data. Again the DH lines showed a broader spread of data, especially in comparison with the BC population, which had only a very narrow distribution (Fig. 13.12). The MPH for plant height showed a more

(a)



(b)

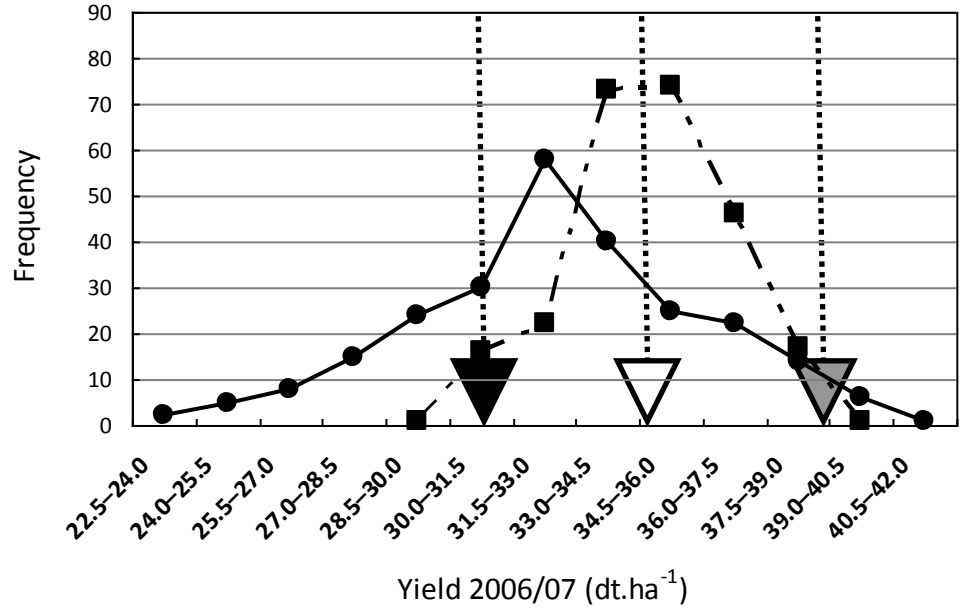


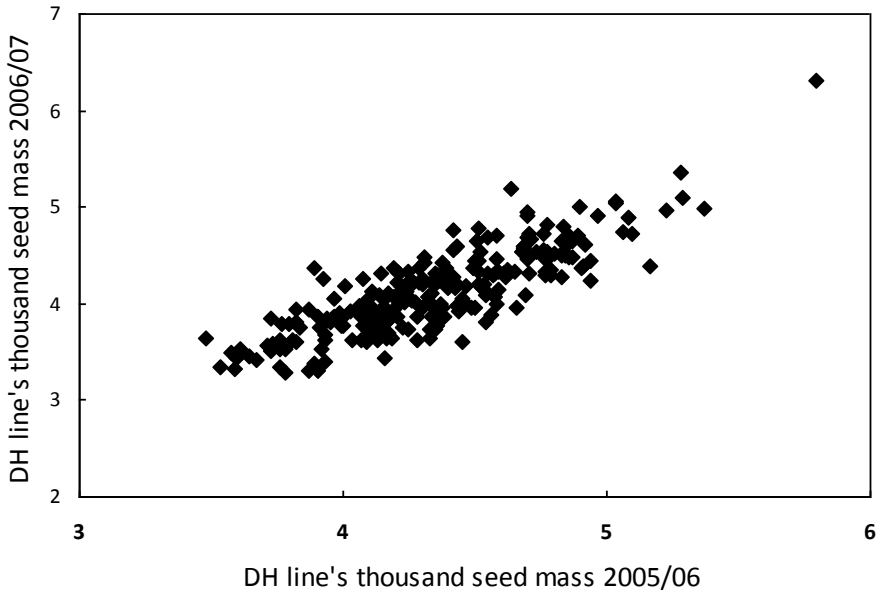
Figure 3.9 Distributions of seed yield in (a) 2005/06 and (b) 2006/07 for 250 DH lines from the population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. Triangles show the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey). Notice the change of position of 'Express 617' in (a) and (b).

consistent pattern in both planting years and was dominated by positive heterosis, although individual accession numbers were not always consistent in both years.

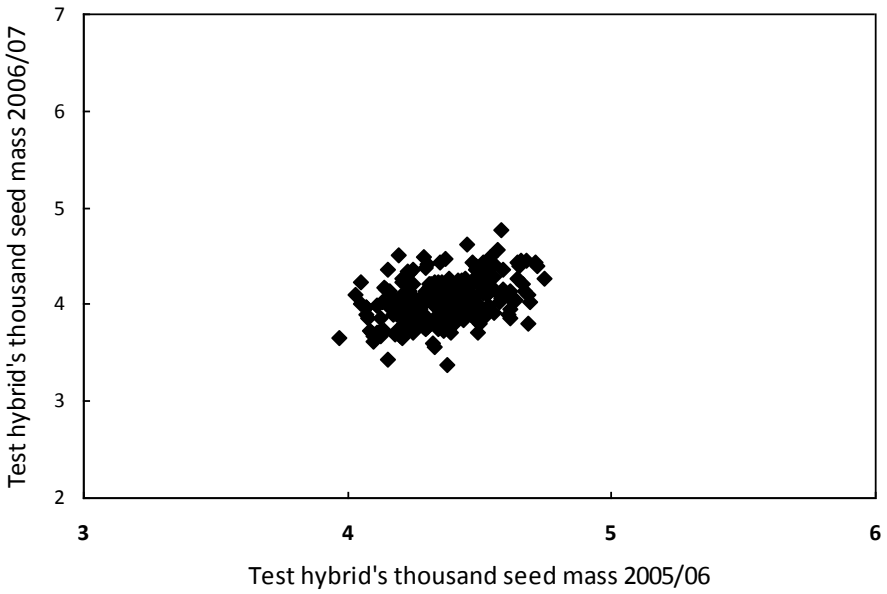
The plant height data distribution patterns of the DH and BC populations during the two planting years again showed that the modus of the BC population shifted slightly to the right compared to the DH population distribution. In both years the range of the BC population was smaller than that of the DH lines. In general, the DH lines in the 2006/07 growing season had a wider range and many individual accessions had lower values than in the earlier year (Fig. 3.13).

The MPH for plant height showed a similar spread of distribution for 2005/06 and 2006/07, however, the correlation was weak between the two years. Only 11 out of 250 (4.4%) accession pairs had negative MPH values in 2005/06 and 22 (8.8%) in 2006/07. The absolute MPH ranged from -1.64 cm (-1.03%, #157) to 17.86 cm (11.84%, #163) in 2005/06, and from -4.9 cm (-3.3%, #171) to 18.3 cm (12.4%, #126) in 2006/07.

(a)



(b)



(c)

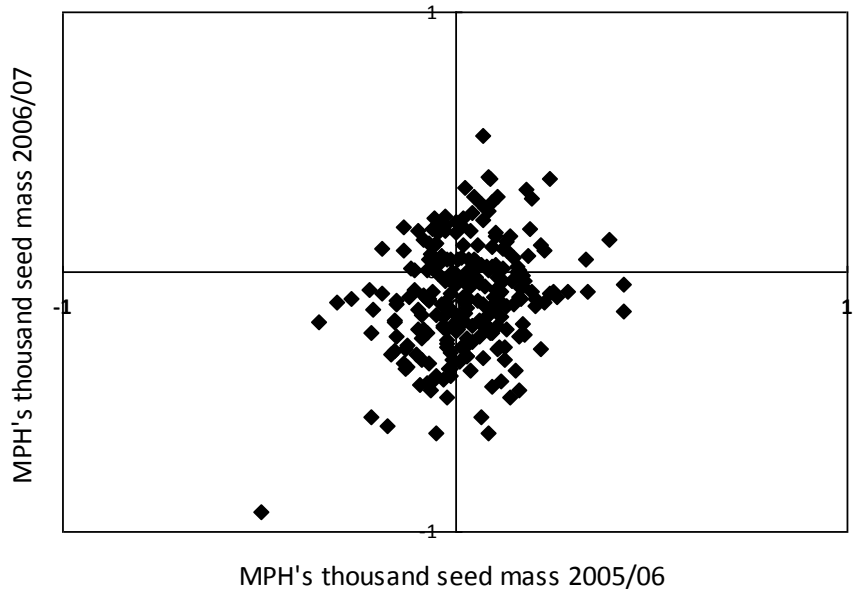
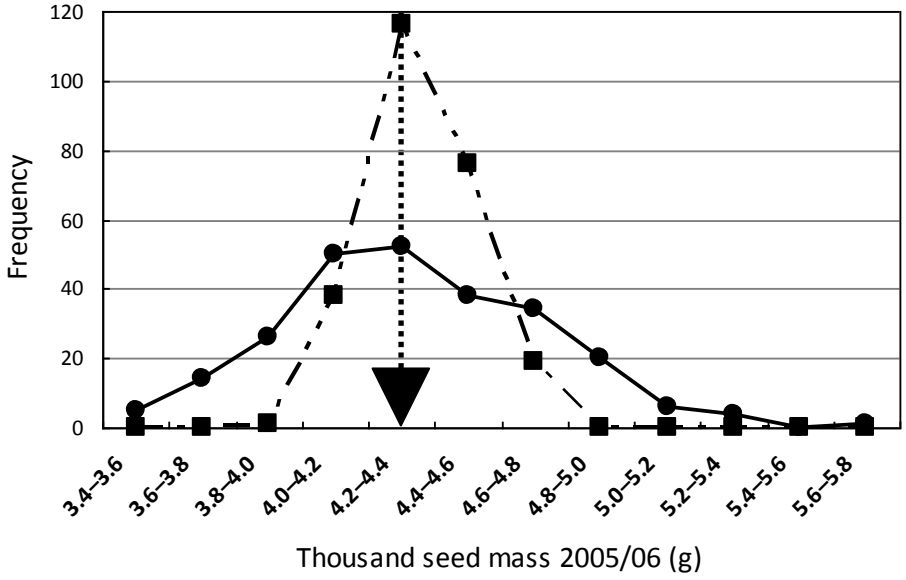


Figure 3.10 Scatter diagrams showing the relationships between thousand seed mass (TSM) in 2005/06 and 2006/07 for (a) 'Express 617' × 'V8' DH lines, (b) their BC test hybrids with 'MSL Express', and (c) mid-parent heterosis (MPH) of the BC test hybrids. Diagrams for the DH and BC populations are made with the same scale to emphasise their relative distributions.

(a)



(b)

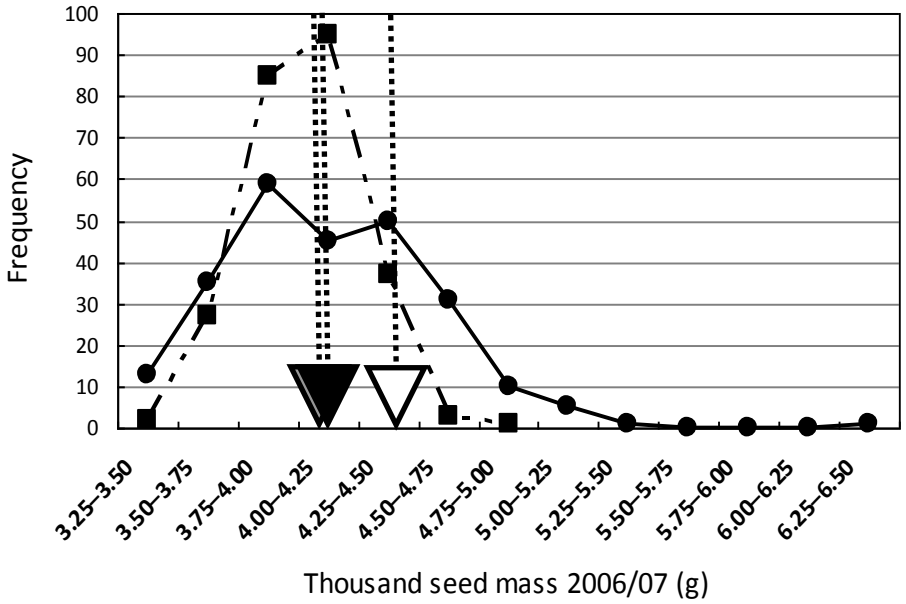
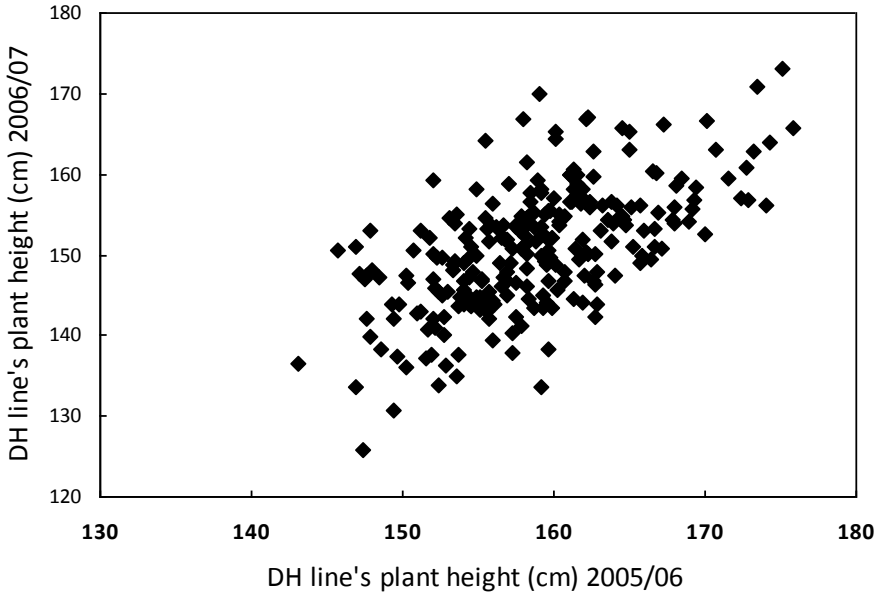


Figure 3.11 Distributions of thousand seed mass (TSM) in (a) 2005/06 and (b) 2006/07 for 250 DH lines from the population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. Triangles show the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey). Notice the different scale of X-axis between (a) and (b).

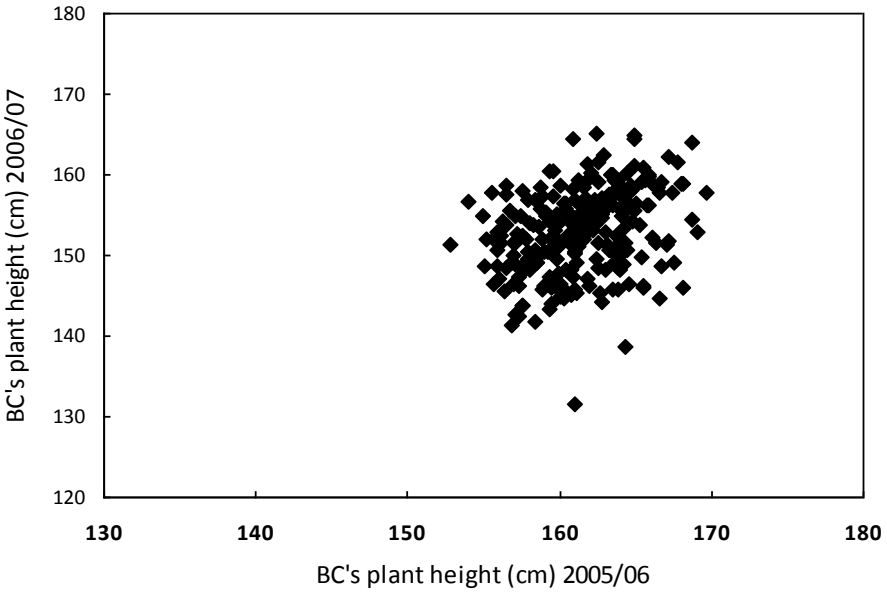
3.4 Phenotypic observations: greenhouse and field trials

As expected due to the complexity of the traits analysed, the correlation analysis on heterosis for traits observed in the greenhouse and field analysis revealed only a few clear relationships. However, some small (lower than 0.2) but still significant correlations existed although all were attributed to yield data from a single growing season, except the correlation between shoot dry weight and yield. The latter had significant but small correlations of shoot dry weight with yield for both the 2005/06 and 2006/07 field trials (both have r = 0.14, see Fig. 3.14) and was of particular interest. Specific leaf weight correlated with two different traits from different planting years (plant height in 2005/06 and yield in 2006/07). Thousand seed mass from both harvest years appeared to be expressed independently from all of the observed early developmental traits.

(a)



(b)



(c)

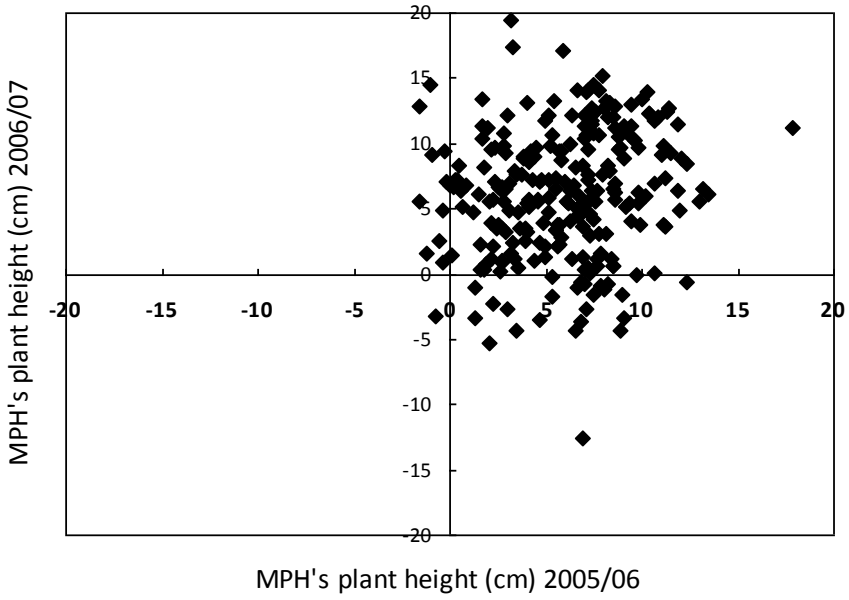
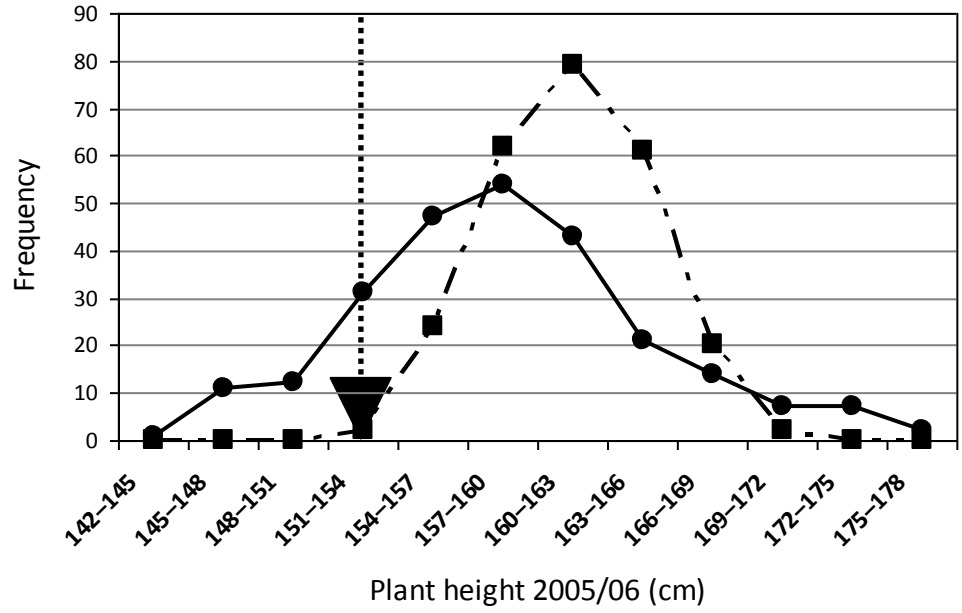


Figure 3.12 Scatter diagrams showing the relationships between plant height in 2005/06 and 2006/07 for (a) 'Express 617' × 'V8' DH lines, (b) their BC test hybrids with 'MSL Express', and (c) mid-parent heterosis (MPH) of the BC test hybrids. Diagrams for the DH and BC populations are made with the same scale to emphasise their relative distributions.

(a)



(b)

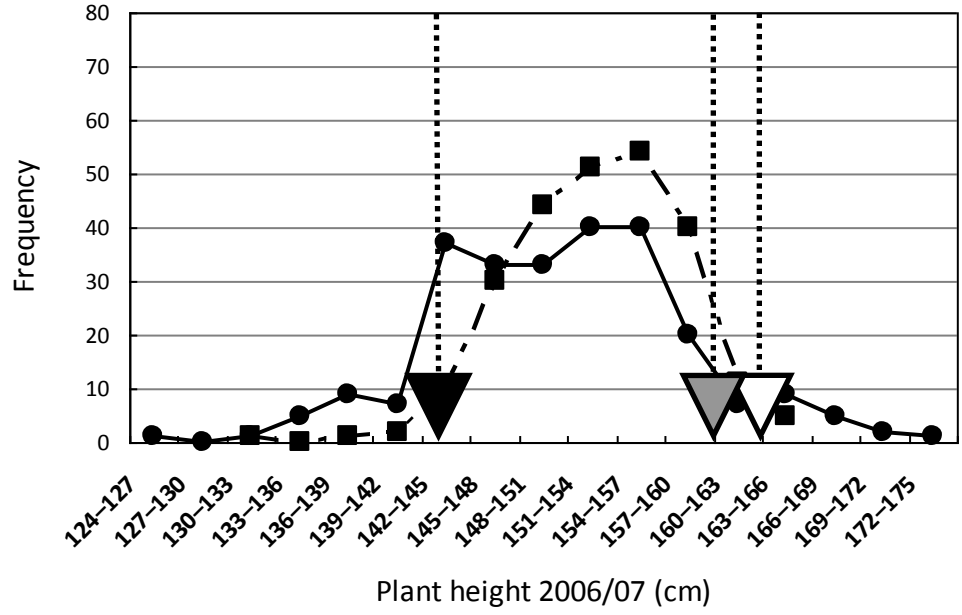


Figure 3.13 Distributions of plant height (in cm) in (a) 2005/06 and (b) 2006/07 for 250 DH lines from the population 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. Triangles show the trait values of the cross parents 'Express 617' (black) and 'V8' (white) and their F1 hybrid (grey). Notice the different scale of axis and the performance of 'Express 617' in the two years.

Since in general there were only low correlations between traits from the greenhouse and field trials, correlation analyses to yield heterosis were repeated with the fifty BC hybrids showing the best and worst heterosis performance, respectively, for the individual early biomass traits. The aim was to to see if hybrid combinations with particularly high or low heterosis for a given early biomass trait also showed correspondingly high or low heterosis for yield. A significant difference ($P > 0.05$) was seen for both harvest years between the seed yield of hybrids with high and low seedling biomass heterosis, respectively (Fig. 3.15). Shoot dry weight was associated with a significant difference in yield heterosis in 2007, whereas hybrids with high and low heterosis for leaf fresh

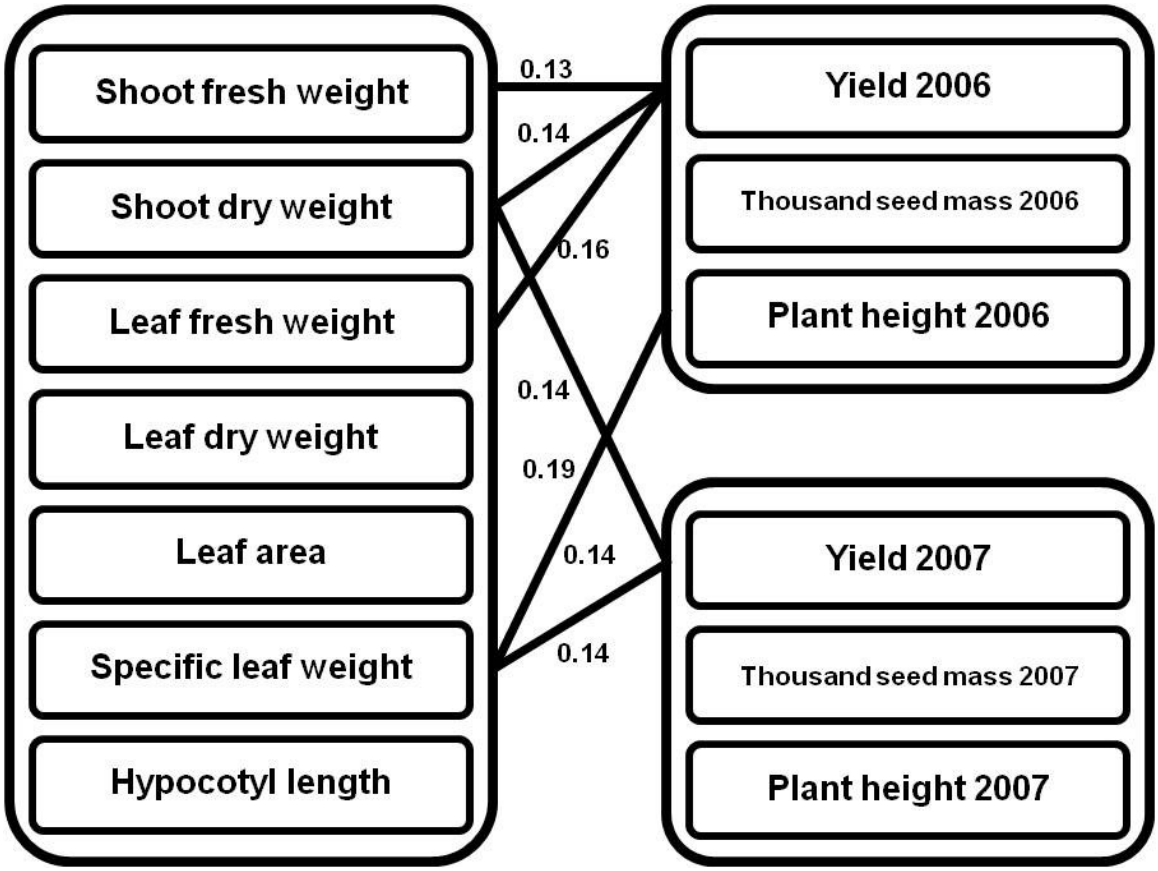
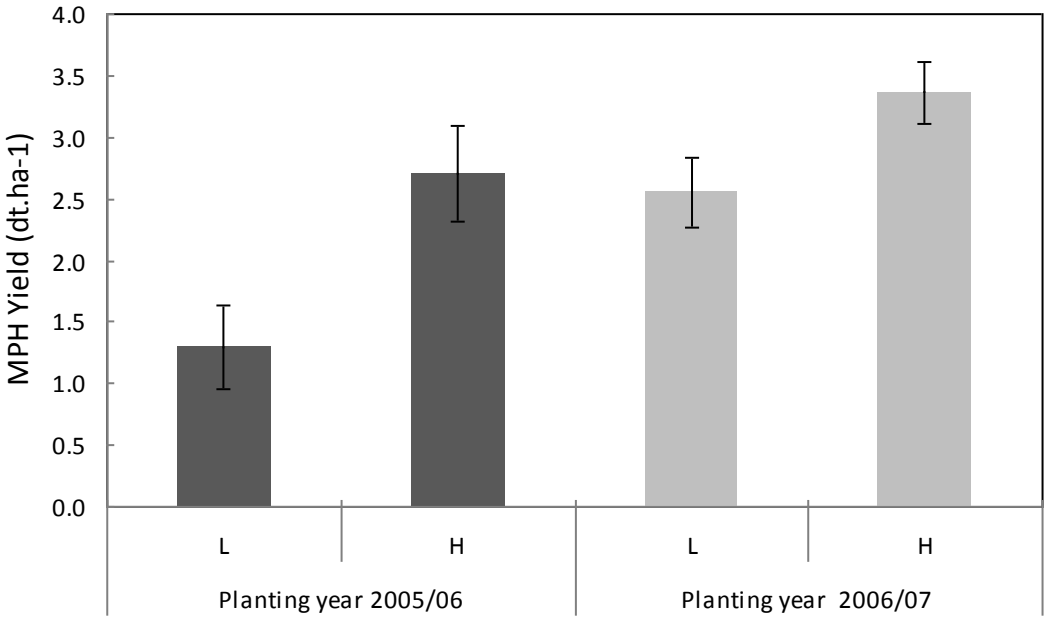


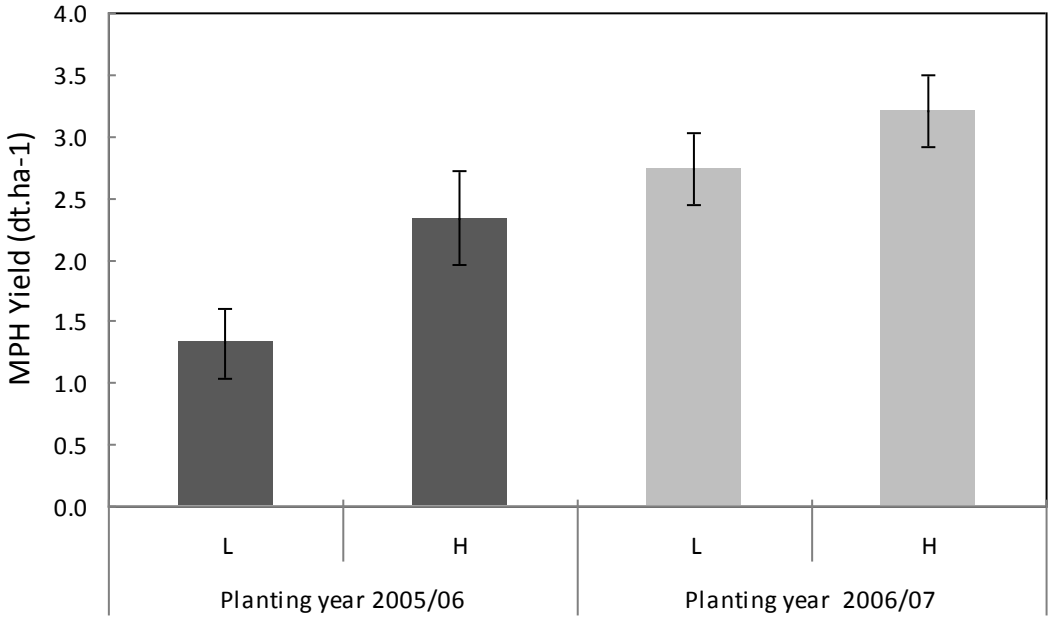
Figure 3.14 Small but significant correlation coefficients (P<0.05) between traits observed in greenhouse and field trials from the mid-parent heterosis (MP) data. Combinations not joined by lines showed no significant correlations.

weight showed significantly different yields at the 2006 harvest. Considering this result and the earlier correlations analysis, and that there were high correlation between shoot fresh weight and shoot dry weight, it appears that there may be a weak but significant relationship between heterosis for shoot weight and heterosis for yield. This could indicate common regulatory mechanisms involved in heterosis in early seedling development and during the seed-filling stages in adult winter oilseed rape plants.

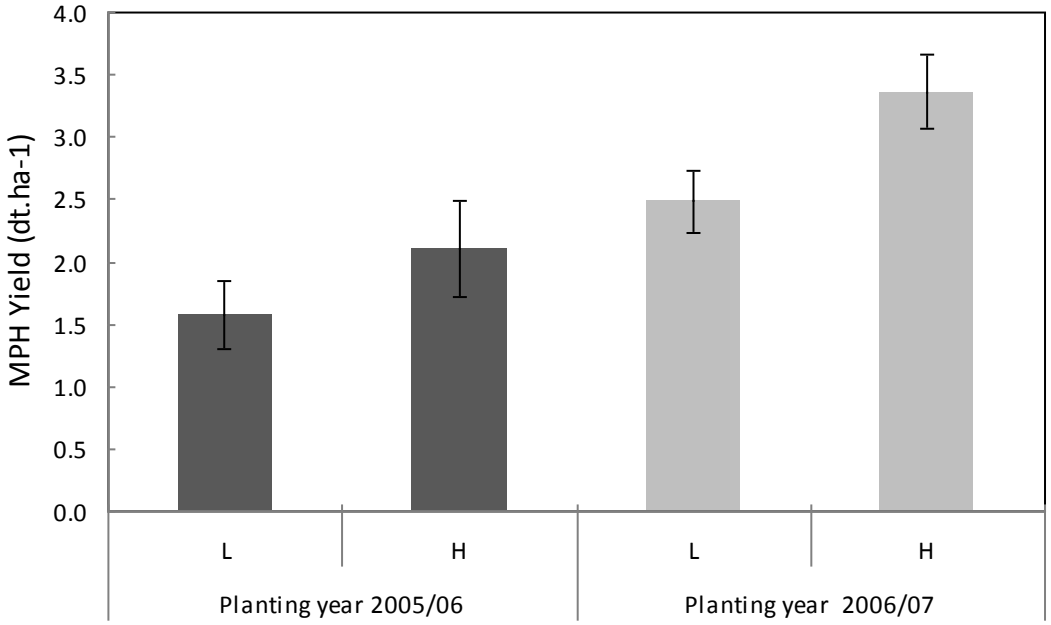
(a)



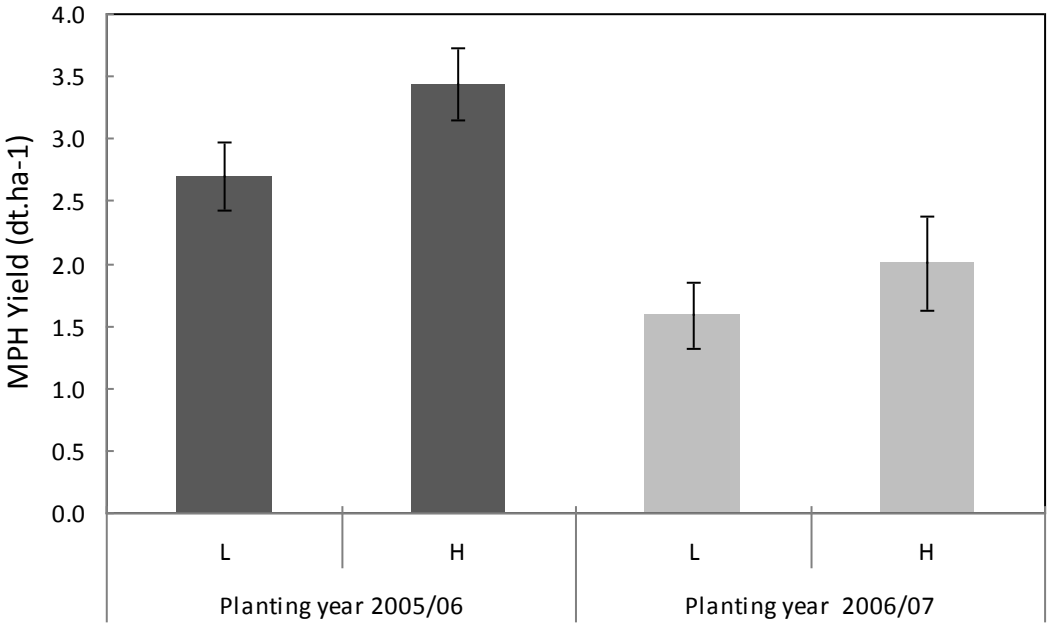
(b)


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MPH Yield (dt.ha-1)
L
H
L
H
Planting year 2005/06
Planting year 2006/07

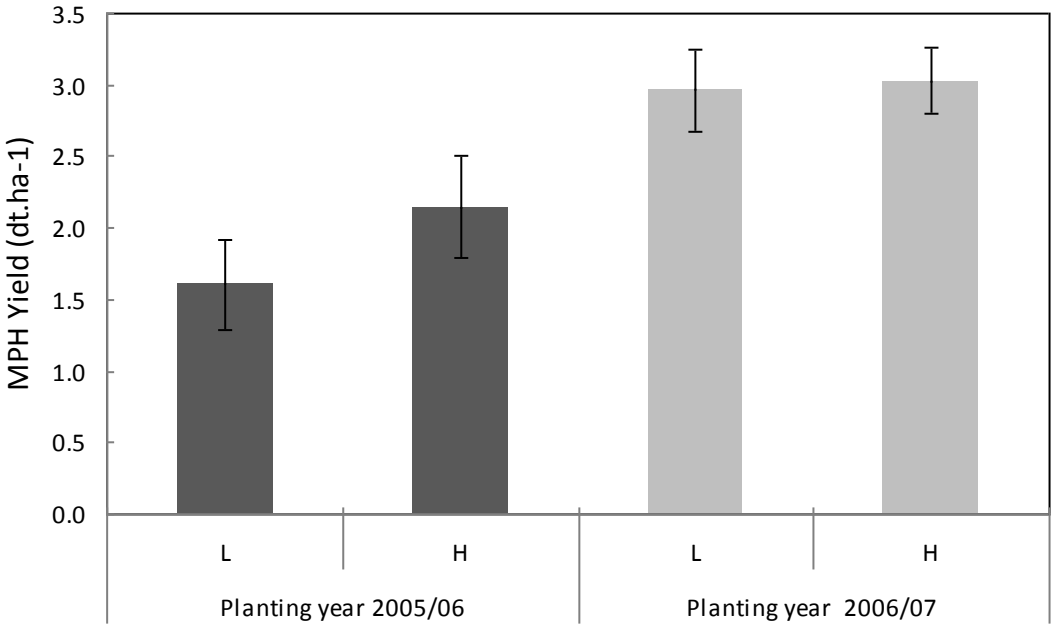
(c)


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MPH Yield (dt.ha-1)
L
H
L
H
Planting year 2005/06
Planting year 2006/07

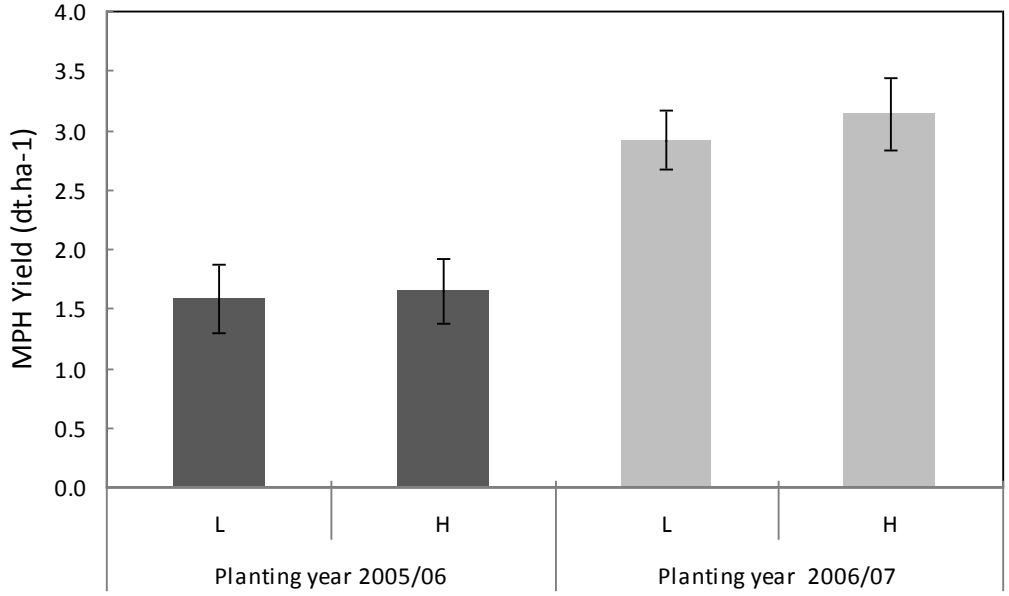
(d)


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MPH Yield (dt.ha-1)
L
H
L
H
Planting year 2005/06
Planting year 2006/07

(e)


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MPH Yield (dt.ha-1)
L
H
L
H
Planting year 2005/06
Planting year 2006/07

(f)



(g)

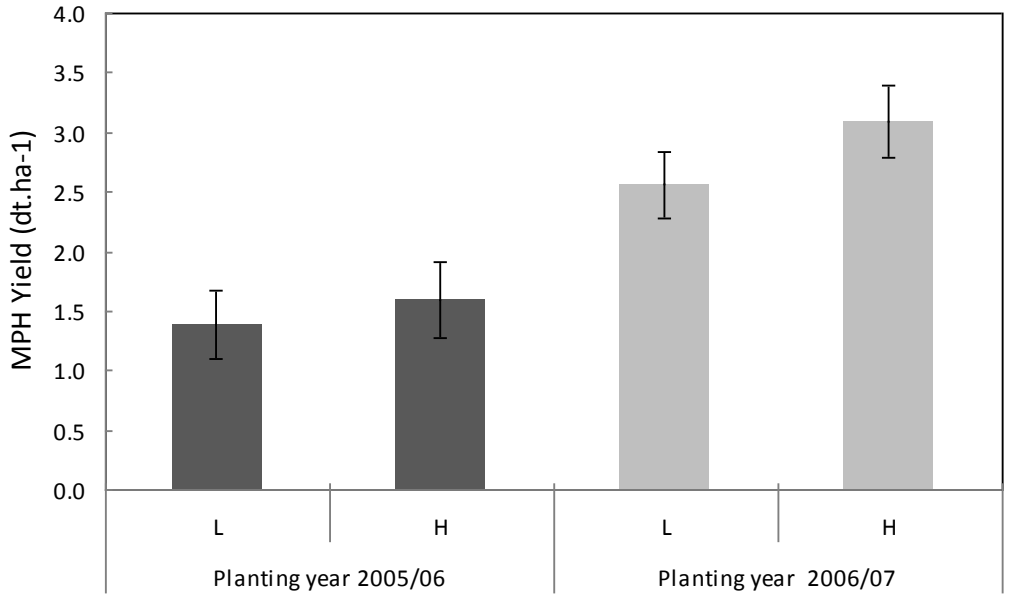


Figure 3.15 Comparison of mid-parent heterosis (MPH) for yield in 2005/06 (dark grey bars) and 2006/07 (light grey bars), respectively, in groups of the 50 BC test hybrids with the respectively highest (H) and lowest (L) MPH for (a) shoot fresh weight, (b) leaf fresh weight, (c) shoot dry weight, (d) leaf dry weight, (e) leaf area, (f) hypocotyl height, and (g) specific leaf weight.

3.5 Quantitative trait loci analysis

As mentioned in Section 2.7, single locus estimates provided by QTL analysis of the MPH data represent dominance effects of the respective QTL, while estimates from the DH data represent additive genetic effects. Incorporating a two-locus interaction model (epistasis model) may give information on additive-to-additive genetic interaction effect when applied to the DH data. Although its genetic interpretation is more difficult, analysis of the BC and MP data sets using an epistasis model is also given to show the possible roles of other types of multilocus interaction.

3.5.1 Main-effect QTL for early morphological traits

From seven traits observed in the greenhouse trial, eleven main-effect QTL were detected. None of these derived from the MPH dataset, while seven and four loci were detected from the DH and BC datasets, respectively (Table 3.1). Percentage of variance due to the QTL compared to the phenotypic variance, as a measure of the relative contribution of the locus, ranged from 6.8% (fresh leaf weight in the BC dataset) to 2.3% (hypocotyl length in the DH dataset). It might be argued that the absence of QTL from the MPH dataset makes it impossible to obtain estimate of dominance of QTL, thus to relate heterosis to any locus. Heterosis effect cannot be attributed to the DH population. On the other hand, the lines provide estimates of additive genetic effects, which are determined directly by alleles they possess and not affected by the genotypes they build. Furthermore, the BC data set gives indications of heterosis effects, since it estimates the *difference* between additive genetic and dominance effects. Therefore, in cases where the BC and DH datasets each show QTL in a common position, a rough approximation of dominance effects on heterosis can be made. This situation, however, was not observed for early biomass traits.

3.5.2 Main-effect QTL of traits observed in field trials

A total of 47 main-effect QTL were observed for plant height at the end of flowering, TSM and yield during the two growing seasons (Table 3.2). Among these, four are QTL from the MPH data set (heterosis) from 2005/06, while seven QTL for heterosis in the

Table 3.1 Early biomass QTL with main effect detected in the greenhouse trial of 250 DH lines from the cross 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. QTL names are derived from the abbreviation of the data set used and the trait observed: D = DH population data, Hb = BC hybrid data, Ht = mid-parent heterosis data, fshw = shoot fresh weight, dshw = shoot dry weight, fphw = photosynthetic organ (leaf) fresh weight, dphw = photosynthetic organ (leaf) dry weight, hch = hypocotyl height, la = leaf area, slw = specific leaf weight. Values of genetic effects are presented after adjustment. Effects of QTL obtained from the DH data set are not adjusted, BC effects are multiplied by *2* and MP effects are multiplied by *-2*.

QTL	Flanking marker		Position (cM)	Range (cM	Effect	Heritability (%)
	Left	Right				
Hbdshw N14	HMR416b	E32M59_142	46.6	37.4–52.2	-0.02	6.0
Hbfshw N14	HMR416b	E32M59_142	44.6	37.4–52.2	-0.21	3.1
Dfphw N11	CB10206	E32M62_248	54.4	39.6–70.1	-0.12	2.6
Dfphw N17	E36M611_10	BRAS014	46.9	36.5–68.8	0.14	3.4
Hbfphw N13	Ol13C10b	E31M55_102	33.4	29.4–43.6	-0.16	6.8
Dhch N03	CB10413	E36M61_83	9.5	0.0–22.5	1.47	2.3
Dhch N05	BRAS063	CB10574	40.8	29.5–49.9	1.61	2.7
Dhch N12	HMR353b	Ni2C12	66.4	61.3–75.8	-1.65	2.9
Dhch N14	Ol10F12	Ol13D02a	15.8	11.8–20.8	-2.01	4.3
Hbla N05	Na12E01b	E31M62_195	53.5	40.8–64.7	-12.84	3.0
Dslw N05	BRAS063	CB10574	32.8	16.4–36.8	-0.04	4.0

various traits were observed in 2006/07. Although these QTL did not overlap between the two years, numerous overlaps were found with QTL from the DH and BC datasets.

Chromosome N07 contained a cluster of QTL from the different datasets exclusively for TSM; one of them is a QTL for heterosis in TSM (Httsm06), whereas other QTL were from the DH and BC datasets for both growing seasons (Table 3.2). Another notable cluster of QTL for TSM (including another heterotic QTL, Httsm07) was located on chromosome N16, accompanied by two QTL for TSM from *per se* data (DH population) plus a QTL for yield heterosis in 2006 (Hty06). Two other clusters of QTL exclusively for TSM were located on chromosomes N09 and N10, respectively, although neither contained a significant heterotic QTL. A further heterotic QTL for TSM was located on chromosome N19.

QTL for yield were found on chromosomes N01, N03, N05, N08, N09, N13 and N16, with heterotic QTL being detected on N03, N08, N13, and N16. As mentioned previously, Hty06 on N16 co-located with a QTL cluster for TSM. A QTL for yield heterosis on N03 (Hty07) co-located with two interaction QTL for TSM and plant height, while a QTL for yield heterosis on N08 (Hty06) co-located with a QTL for yield in the BC dataset (Hby08) and two QTL for epistatic interactions affecting hypocotyl length whose respective pairs also co-located at the same position. Based on these findings it is possible that these QTL have pleiotropic effects, or that they may represent regulatory gene loci involved in numerous developmental processes.

QTL for plant height were localised on a number of chromosomes. Three of these QTL were heterotic, namely on chromosomes N03 and N05 in 2006/07 and on chromosome N11 in 2005/06. The QTL on N05 for heterosis in plant height (Htph07) co-localised with a QTL for *per se* yield (Dy07). Two further QTL for *per se* plant height were detected in both years on N16 and N13.

3.5.3 Active regions

Numerous clusters were observed where QTL for various traits from one or more of the datasets occurred in a common location. For the sake of simplicity the term “active region” was introduced in this work to describe these regions. An active region is any

Table 3.2 QTL with main effect detected in the field trials of 250 DH lines from the cross 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'. QTL names are derived from the abbreviation of the data set used and the trait observed: D = DH population data, Hb = BC hybrid data, Ht = mid-parent heterosis data, y = yield, ph = plant height, tsm = thousand seed mass. Values of genetic effects are presented after adjustments for BC and MP: Effects of QTL obtained from the DH data set are not adjusted, BC effects are multiplied by 2 and MP effects are multiplied by -2.

QTL	Flanking marker		Position (cM)	Range	Effect	Heritability (%)
	Left	Right				
Dph07 N01	E44M47_77	E45M574_23	60.8	54.8–68.6	-1.30	3
Dph07 N07	E39M49_307	E44M58_101	6.0	0.0–16.0	-1.99	6
Dph07 N13	BRAS039b	E36M51_45	91.1	84.9–96.5	-2.66	11
Dph07 N16	E31M49_112	E32M51_350	90.2	83.8–97.8	-3.02	14
Hbph07 N12	E43M51_140	CB10316	44.0	35.6–54.8	2.12	4
Hbph07 N16	E45M48_404	E32M59_285	111.7	97.8–122.1	-2.00	4
Htph07 N03	CB10079	E43M51_254	133.0	123.6–135.0	1.56	3
Htph07 N05	BRAS063	CB10574	34.8	19.5–51.5	1.60	3
Dy07 N05	BRAS063	CB10574	36.8	32.8–42.8	-1.41	15
Dy07 N09	E42M55_131	E31M55_249	85.4	79.6–89.4	-1.16	10
Hby07 N05	E31M62_195	E42M55_166	58.7	53.5–64.7	-1.28	11
Hty07 N03	Na14G10	E42M55_125	61.2	52.8–67.2	-0.78	4
Hty07 N13	Ol10E05	HMR320	2.3	0.0–8.0	-0.60	3
Dtsm07 N07	GMR166	Ra2G08	24.2	22.2–28.6	-0.20	23
Dtsm07 N09	E44M51_350	E35M60_540	17.2	7.7–21.9	0.11	6

Table 3.2 Continued

QTL	Flanking marker		Position (cM)	Range	Effect	Heritability (%)
	Left	Right				
Dtsm07 N10	Na12H04	E42M55_595	74.2	70.3–78.2	-0.12	9
Dtsm07 N16	E34M54_45	E32M51_225	68.5	64.5–72.5	0.13	10
Hbtsm07 N07	Ra2G08	Na12B02	27.1	16.4–34.6	-0.16	10
Hbtsm07 N09	Na14C12	E36M57_123	57.4	49.5–64.0	0.14	8
Hbtsm07 N10	Na12H04	E42M55_595	74.2	56.4–78.2	-0.10	4
Httsm07 N06	E45M49_137	E34M55_290	66.3	59.5–74.0	-0.08	3
Httsm07 N16	E34M54_45	E32M51_225	70.5	55.3–94.2	-0.08	4
Httsm07 N19	Ol12F07	E32M49_386	96.4	87.6–109.6	0.12	7
Dy06 N05	CB10609	Na12E01b	47.9	42.8–55.5	-0.85	5
Dy06 N16	CB10211b	BRAS048	130.5	116.1–132.5	1.12	9
Hby06 N01	CB10097	E34M51_63	22.4	16.9–26.4	-0.76	3
Hby06 N05	E34M59_94	BRAS063	29.5	23.5–40.8	-1.12	6
Hby06 N08	CB10629	E43M62_222	45.1	38.9–51.4	-0.80	3
Hby06 N09	Na12E06B	Na14C12	49.5	46.0–53.4	-0.86	4
Hty06 N08	CB10629	E43M62_222	41.1	38.9–49.4	-0.90	5
Hty06 N16	E34M54_45	E32M51_225	74.5	66.5–83.8	-0.70	3
Dtsm06 N07	GMR166	Ra2G08	24.2	22.2–27.1	-0.17	19
Dtsm06 N09	E44M51_350	E35M60_540	15.2	7.7–19.9	0.11	8
Dtsm06 N09	MR230	CB10116B	141.0	137.0–145.9	0.09	5

Table 3.2 Continued

QTL	Flanking marker		Position (cM)	Range	Effect	Heritability (%)
	Left	Right				
Dtsm06 N10	Na12H04	E42M55_595	76.2	72.2–78.2	-0.11	8
Dtsm06 N16	E34M54_45	E32M51_225	70.5	66.5–75.9	0.12	9
Hbtsm06 N07	Na12B02	Na12E11	30.6	28.6–34.6	-0.11	12
Hbtsm06 N09	E44M51_350	E35M60_540	13.2	7.7–17.9	0.10	10
Hbtsm06 N09	E43M62_336	MR230	126.3	110.6–134.3	0.10	10
Hbtsm06 N10	Na12E09	Ol10B11	59.9	58.4–67.9	-0.08	7
Hbtsm06 N16	E33M49_199	Na12E01a	53.3	45.6–58.3	0.09	8
Httsm06 N07	GMR166	Ra2G08	24.2	18.4–28.6	0.09	12
Dph06 N10	E34M62_109	E46M59_241	9.3	4.0–17.3	0.93	2
Dph06 N13	E46M62_120	BRAS039B	86.9	73.9–95.1	-1.24	4
Dph06 N16	E31M49_112	E32M51_350	88.2	85.8–92.2	-3.19	25
Hbph06 N16	E31M49_112	E32M51_350	88.2	83.8–94.2	-1.98	8
Htph06 N11	E31M55_102	E32M54_52	43.6	39.6–48.5	1.48	5

region in a chromosome that possesses at least one main-effect QTL from one of the datasets. If two or more overlapping QTL were found in the same region, this was treated as a single active region.

A total of 26 active regions were detected on 15 of the 19 chromosomes. Only N02, N04, N15, and N18 did not have active regions, although they did have epistatic QTL, of which some interacted with QTL located in active regions. Chromosome N09 had the most active regions, namely five.

Active regions containing more than one QTL were found in chromosomes N05, N07, N08, N09 (two regions), N10, N11, N13, N14 and N16, whereby some regions deserved particular attention. Chromosome N05 has active region *qN05_1* spreading from 16.4 cM to 64.7 cM. This long region is associated with field traits (yield *per se* in both planting years and heterosis for 2007 plant height) and greenhouse traits (SLW, leaf area and hypocotyl length). Chromosomes N07, N09 and N10 possessed active regions exclusively related to thousand seed mass (TSM) for both planting years, especially *qN07_1* (which also influenced heterosis in 2006), *qN09_1* and *qN10_2*. Other regions related to TSM were *qN06_1* (influence on heterosis in 2007), *qN09_2* (2007, shared with yield 2006), *qN09_4* and *qN09_5*, *qN16_1*, and *qN19_1* (heterosis in 2007). Region *qN08_1* had two QTL for yield in 2006 from both the BC and MP data sets. Another particularly interesting active region was *qN14_2*, which contained QTL from the BC data set for both shoot dry weight and fresh weight. Two overlapping QTL for plant height in both years were located in region *qN13_3*. These regions, in which main effect QTL affected multiple traits, appear to influence different traits throughout the lifespan of plant, and could represent regulatory or "housekeeping" genes. Single-trait regions, on the other hand, such as for thousand seed mass, are more likely to encode specific regulators of the trait in question that are not involved in other traits.

Other active regions had only single main-effect QTL; however these are treated as equally important because interacting QTL – from any trait – may indicate a connection between active regions. For example, the region *qN10_1* (with a main-effect QTL for plant height) interacted with the active region *qN13_3* with regard to hypocotyl length (Figure 3.16), and the latter is an active region for two main-effect QTL influencing plant height. Region *qN10_1* was also related through a QTL for yield heterosis with *qN05_1*, an active region containing QTL for yield and plant height. This interconnection suggested a possible co-regulatory function among the respective regions and their corresponding QTL, including a regulatory effect influencing yield heterosis.

3.5.4 Epistatic QTL

A considerable degree of two-locus epistatic interaction was observed. Many additive to additive epistatic QTL could be detected in the DH dataset (see Section 2.7; Appendix C). Genetic interpretation of interaction terms estimated by each data set is complex due

to the large quantity of epistatic interactions; hence the interactions were classified into three classes for each dataset: Class I – interaction between two main effect QTL, Class II – interaction between main effect QTL and non-main effect QTL, and Class III – interaction between two non-main effect QTL. A similar classification was made based not on QTL but on active regions. Tabular summaries of the epistatic interaction classes for QTL and active regions are presented in Table 3.3 to Table 3.6.

A notable feature of the detectable epistatic interactions was that Class I interactions were almost non-existent in both the field and greenhouse trials. Using QTL as the basis of grouping provided no main-effect QTL to main-effect QTL interaction. Furthermore, there were also very few Class II QTL interactions, so that Class III interactions were the dominating class of epistasis detected. Radoev et al. (2008b) found a similar situation using a closely-related mapping population. This indicated that main-effect QTL did not

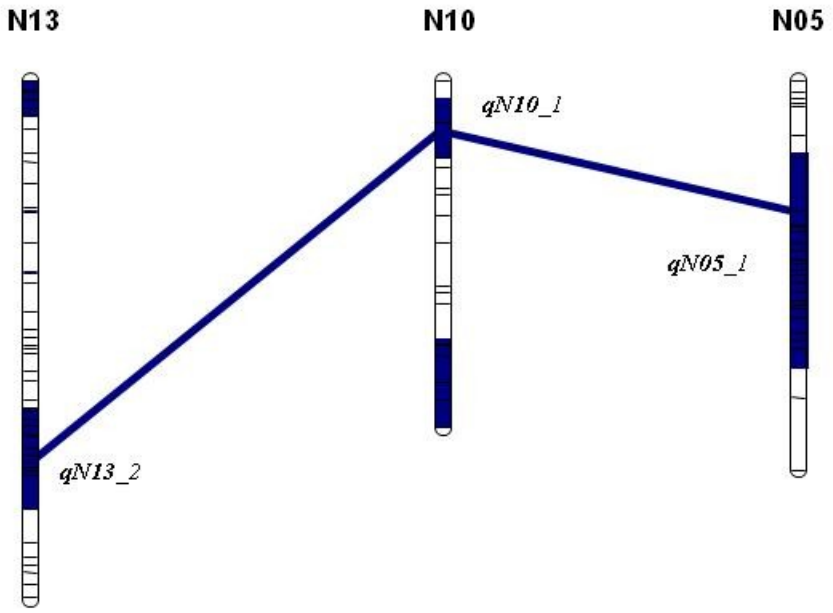


Figure 3.16 Interconnection among active regions in chromosomes N05, N10, and N13. Regions *qN10_1*, *qN13_2* and *qN05_1* each had QTL for plant height. An interacting QTL for hypocotyl length connected *qN10_1* to *qN13_3* and another interacting QTL for yield heterosis connected *qN10_1* to *qN05_1*. The latter also had QTL for yield.

Table 3.3 Number of epistatic interactions among QTL for traits observed in the field trials in 2005/06 and 2006/07. Type I = interaction between two main-effect QTL, Type II = interaction between a main-effect QTL with and a non-main effect QTL, Type III = interaction between two non-main effect QTL.

Harvest time	Trait	Data set	Interaction type			
			Type I	Type II	Type III	Total
2006	Yield	DH	0	0	5	5
		BC	0	0	5	5
		MP	0	0	3	3
	Plant height	DH	0	0	10	10
		BC	0	1	9	10
		MP	0	0	2	2
	TSM	DH	0	0	6	6
		BC	0	0	5	5
		MP	0	0	2	2
2007	Yield	DH	0	0	1	1
		BC	0	0	8	8
		MP	0	0	8	8
	Plant height	DH	0	0	7	7
		BC	0	1	6	7
		MP	0	1	5	6
	TSM	DH	0	1	7	8
		BC	0	0	2	2
		MP	0	0	3	3
Total per population		DH	0	1	36	37
		BC	0	2	35	37
		MP	0	1	23	24
Total			0	4	94	98

play a major role in epistatic interactions related to heterosis, whereas complex interactions between non-main effect QTL appear to be heavily involved in expression of heterosis for different biomass and yield traits.

Table 3.4 Number of epistatic interactions among active regions for traits observed in the field trials in 2005/06 and 2006/07. Type I = is interaction between two main-effect QTL, Type II = interaction between a main-effect QTL with and a non-main effect QTL, Type III = interaction between two non-main effect QTL.

Year	Trait	Data set	Interaction type			
			Type I	Type II	Type III	Total
2006	Yield	DH	1	2	2	5
		BC	0	1	4	5
		MP	2	1	0	3
	Plant height	DH	1	3	6	10
		BC	0	6	4	10
		MP	0	2	0	2
	TSM	DH	0	3	3	6
		BC	0	2	3	5
		MP	0	1	1	2
2007	Yield	DH	0	0	1	1
		BC	0	5	3	8
		MP	2	4	2	8
	Plant height	DH	1	2	4	7
		BC	2	2	3	7
		MP	1	4	1	6
	TSM	DH	0	3	5	8
		BC	1	1	0	2
		MP	0	0	3	3
Total per population		DH	5	10	20	35
		BC	3	18	17	38
		MP	2	11	7	20
Total			10	39	44	93

Further investigations showed that certain interactions involving the same trait could be found clustering in the same location. This finding is quite interesting, since such patterns were typical for transcription factors. Many investigations on the nature of QTL for complex traits have led to a conclusion that the underlying genes were transcription

Table 3.5 Number of epistatic interactions among QTL for early biomass traits observed in the greenhouse trials. Type I = is interaction between two main-effect QTL, Type II = interaction between a main-effect QTL with and a non-main effect QTL, Type III = interaction between two non-main effect QTL.

Trait	Data set	Interaction type			
		Type I	Type II	Type III	Total
Fresh shoot weight	DH	0	0	3	3
	BC	0	0	6	6
	MP	0	0	5	5
Dry shoot weight	DH	0	0	5	5
	BC	0	0	3	3
	MP	0	0	1	1
Fresh leaf weight	DH	0	1	5	6
	BC	0	0	4	4
	MP	0	0	4	4
Dry leaf weight	DH	0	0	3	3
	BC	0	0	4	4
	MP	0	0	2	2
Leaf area	DH	0	0	3	3
	BC	0	0	2	2
	MP	0	0	4	4
Specific leaf weight	DH	0	0	1	1
	BC	0	0	0	0
	MP	0	0	1	1
Hypocotyl length	DH	0	0	6	6
	BC	0	0	7	7
	MP	0	0	2	2
Total per population	DH	0	1	26	27
	BC	0	0	26	26
	MP	0	0	19	19
Total		0	1	71	72

factors (see, for instance, Yano et al. 2000a, 2000b, Gibert et al. 2005). Since the investigation of interactions is a demanding task, clusters of interacting QTL are a useful starting point to identify target regions where relevant transcription factors might be located.

Table 3.6 Number of epistatic interactions among active regions for traits observed in the greenhouse trials. Type I = is interaction between two main-effect QTL, Type II = interaction between a main-effect QTL with and a non-main effect QTL, Type III = interaction between two non-main effect QTL.

Trait	Data set	Epistasis			
		Type I	Type II	Type III	Total
Fresh shoot weight	DH	0	2	1	3
	BC	2	1	3	6
	MP	0	3	2	5
Dry shoot weight	DH	0	2	3	5
	BC	0	2	1	3
	MP	1	0	0	1
Fresh leaf weight	DH	0	3	3	6
	BC	1	2	1	4
	MP	2	2	0	4
Dry leaf weight	DH	1	1	1	3
	BC	1	1	2	4
	MP	1	1	0	2
Leaf area	DH	0	0	3	3
	BC	0	1	1	2
	MP	1	1	2	4
Specific leaf weight	DH	0	0	1	1
	BC	0	0	0	0
	MP	0	1	0	1
Hypocotyl length	DH	0	2	4	6
	BC	1	3	3	7
	MP	1	1	0	2
Total per population	DH	1	10	16	27
	BC	5	10	11	26
	MP	6	9	4	19
Total		12	29	31	72

3.5.5 Epistatic QTL considering active regions

Considering active regions, which meant treating a cluster of main QTL as a single QTL region, changed the composition of QTL interaction classes (Table 3.4 and 3.6). In particular this led to an increase in the number of detectable Class I and Class II interactions for all the data sets.

In the field trial data, Class I interactions increased from zero to almost 11% and Class II interactions from 4% to 43%, while the Class III interactions were consequently reduced from 96% to 46%. In the MP data set, in which a total of 20 interactions were observed, Class I interactions were 21% higher (from 0%), Class II increased from 4% to 50% and Class III reduced from 96% to 29%. The use of active regions, instead of strict-to-trait epistatic interaction was a useful reminder that a looser definition of complex traits could help gaining more information on QTL-to-QTL interactions.

Similar shifts were also observed in the greenhouse trial. Class I interactions increased from 0 to 12 (17%) and Class II interactions from 1 (1%) to 29 (40%). Consequently Class III interactions decreased from 99% to only 43%. When we look at the interaction QTL from the MP data set, a 32% increase (from 0 to 6) of Class I interactions and a 47% increased (from 0 to 9) of Class II interactions QTL were detected.

Considering common active regions for field trial data as well as greenhouse trial data demonstrated that treating complex traits without a strict discrimination between different traits traits can give valuable additional information. Although few statistically significant average-based correlation coefficients were observed between traits in the two experiments, at the same time numerous interacting QTL were observed among statistically uncorrelated traits. This suggests potential “minor” interactions that could not be detected at a phenotypic level, and may be a further indication of factors with diverse influences on various traits. This gives also indication of the high complexity of the genetic architecture behind each observed trait.

3.6 Genetic action of the QTL

Due to the genetic structure of the DH and BC populations used in this study, additive genetic, dominance, and epistatic effects can be independently estimated. With regard to heterosis we can only use information on dominance as an effect that is purely estimated (using the MP formula). Estimations derived from the BC population, as well as interactions derived from the BC population data or the MP formula, are functions that are difficult to interpret. However, they can still be used to a certain extent.

3.6.1 Greenhouse trial

From the greenhouse trial, which was aimed at early morphological traits, there were no QTL with dominance effect related to heterosis, although four QTL showed significant differences between additive genetic and dominance effects as estimated using data from the BC population (Table 2.1). Two of these QTL were located in the same region of N14 and derived from two closely related traits (dry and fresh shoot weight). The other two QTL were for fresh leaf weight (on N13) and for leaf area (on N05). These QTL had a low contribution to phenotypic variation (ranged from 3.0 to 6.0%) and their effect had a negative sign, indicating that the dominance effect was higher than the additive genetic effect. This was in agreement with the phenotypic observation that the BC population tended to have slightly higher values than the DH, thus indicating the advantage of heterozygotes.

When we compare the QTL effects of traits from the BC population to BC average value, their effects were relatively small (Table 3.7), i.e. lower than 5%. However, the effect of QTL calculated from BC is actually only half the difference between additive and dominance effects. If these QTL effects are compared to the *difference* between DH and BC population means, we can see that these QTL really have a high relative contributions. They reach reach almost 20% for fresh shoot weight and more than 80% for leaf area.

Interacting QTL derived from the BC and MP data sets were abundant in the greenhouse trial. All the traits observed had interacting QTL which contribution mostly around 6% to

Table 3.7 Genetic effects of main-effect QTL found from the the BC test hybrids data set in the greenhouse trials. The effects are estimated from the BC population relative to the BC population average and to the average difference between the BC and DH population means. A negative effect value indicates that materials with the allele from parent 'Express 617' have a lower average than those with the corresponding allele from parent 'V8'.

Trait	Location	Effect	BC population average	Relative effect to BC pop. average (%)	Average difference	Relative effect to average diff. (%)
Dry shoot weight	N14	-0.02	0.77	-2.6	-0.05	40.0
Fresh shoot weight	N14	-0.21	13.26	-1.6	-1.11	18.9
Fresh leaf weight	N13	-0.16	5.58	-2.9	-0.34	47.1
Leaf area	N05	-12.84	290.30	-4.4	-15.51	82.8

7% to phenotypic variation and a maximum of of 14.7% (*Appendix C*). The type of interaction contributing to the variation is unknown in this case, since we cannot distinguish additive-to-additive from additive-to-dominance or dominance-to-dominance interactions. Nevertheless, the estimates were functions of purely epistatic genetic components. Thus, they provided evidence that QTL interactions play an important role in determining heterosis in the early phase of oilseed rape development.

3.6.2 Field trials

Unlike the greenhouse experiment, QTL that directly estimated dominance effects were found for traits in field trials. Both planting years resulted in series of QTL showing

Table 3.8 Co-location of heterotic main-effect QTL with epistatic QTL of the same or other traits.

Chromosome	Main QTL	Co-locating epistatic QTL	Counterpart chromosome of the epistatic QTL
N01	Hby06	Hty07	N13
N03	Hty07	Hbtsm07	N16
		Hbph07	N07
N05	Htph07, Hby06	Htph07	N04
		Htfshw	N08
		Hbfphw	N14
	Hbla, Hby07	Hbdshw	N15
		Htla	N15
		Hbph06	N12
		Hty06	N10
N06	Httsm07	Hbph06	N11
N07	Httsm06, Hbtsm07,	Hthch	N19
	Hbtsm06	Hbph07	N03
N08	Hty06, Hby06	Hbhch	N10
		Hthch	N10
N09	Hby06, Hbtsm07	Hbhch	N02
N10	Hbtsm06, Hbtsm07	Hbhch	N08
		Hthch	N08
		Htph06	N06
		Hty07	N16
		Hby06	N09
		Htph07	N01
N11	Htph06	Hbph06	N13
		Htph07	N16

Table 3.8 Continued

Chromosome	Main QTL	Co-locating epistatic QTL	Counterpart chromosome of the epistatic QTL
N13	Hty07	Hbdshw	N06
		Hbfshw	N19
		Hbph07	N17
		Hty06	N07
N16	Hty06, Httsm07	Htfshw	N13
		Hbtsm07	N03
N19	Httsm07	Hbph07	N06

additive genetic, dominance and two-locus interaction effects. All the traits observed in both planting years gave at least one QTL related to a dominance effect (see Table 3.2). QTL with dominance-related epistatic effects, derived from the BC and MP data sets, were abundant; some even co-located with main-effects QTL. The latter could be found in N05 (for plant height heterosis 2007), N09 (for thousand seed mass 2007, albeit from the BC and DH data sets) and N16 (for plant height 2007 BC). From the DH data set there were two such cases, i.e. at N11 (for fresh leaf weight) and N17 (for fresh leaf weight), although neither QTL overlapped with QTL involved in heterosis.

When QTL for all traits were considered together, the co-location of main-QTL with epistatic-QTL became common (Table 3.8). These might indicate pleiotropism, where heterosis-related genes do not influence only a single trait but rather many traits simultaneously through some common upstream or regulatory process. Some of the relationships showed interesting nature, by relating certain main QTL through interacting QTL for different traits. Examples of this were observed between two main-effect QTL on N08 and N10, and between main-effect QTL on N03 and N16.

It was not easy to thoroughly conclude whether a heterosis-relevant main-effect QTL showed partial or complete dominance, as well as overdominance, since most of main-effect QTL showing heterosis did not co-locate at the same position with other main-effect QTL from the same trait that had additive effect (from DH data set). An estimate of additive effects at a specific locus is necessary to ascertain the type of dominance effect.

Table 3.9 Estimates of additive genetic effects ($\hat{a}$) and dominance effects ($\hat{d}$) based on co-locating QTL from different datasets and their actions. Italicised figures are obtained from derivation using the other estimates. PD = partial dominance.

Chromo-some	**Trait**	$\hat{a}$	$\hat{d}$	**Action**
N07	Thousand seed mass 2005/06	-0.17	0.09	PD to 'V8'
N07	Thousand seed mass 2006/07	-0.20	*-0.04*	PD to 'Express'
N08	Yield 2005/06	*-1.7*	-0.90	PD to 'Express'
N09	Thousand seed mass 2005/06	0.11	*0.01*	weak PD to 'Express'
N10	Thousand seed mass 2006/07	-0.12	*-0.02*	weak PD to 'Express'
N16	Plant height 2005/06	-3.19	*-1.21*	PD to 'Express'
N16	Thousand seed mass 2006/07	0.13	-0.08	PD to 'V8'

For example, in the active region *qN07_1* on N07, a heterotic QTL (from the MP data set) for TSM co-located with an additive QTL for TSM. At the same active region, two main QTL for TSM from the BC and DH data sets also co-located. Similar MP and DH combinations appeared again on the homoeologous chromosome N16. A BC and DH pairing also appeared on the homoeologous chromosomes N09 (*qN09_1* region) and N10 (*qN10_2* region), again for TSM. In addition, there was a BC-DH pairing for plant height found at N16.

From the estimates of additive genetic and dominance effects obtained from co-locating QTL from different datasets (Table 3.9), all the QTL invariably gave partial dominance effect. From seven QTL which both additive and dominant effect could be estimated, only two QTL had dominance direction in favor of 'V8'. This was largely in agreement with the direct measurements (Fig. 3.11) of the line parental lines and the hybrid, in which the hybrid was in favor of 'Express' although the latter was undermined by 'V8' in

performance. A QTL for TSM measured in 2005/06 and a QTL for TSM in 2006/07 were located in the same active region but not co-located. They had different dominance direction. Unfortunately, we could not compare results from 2005/06 planting year with phenotypic measurement since 'V8' was not involved. Plant height in 2005/06 had partial dominance QTL, whereas yield of 2005/06 had a partial dominance QTL, too, which also could not be compared with the phenotypic situation. To compare the results of 2005/06 with 2006/07 is problematic, since yields and overall performance of was clearly lower in 2006/07 compared to 2005/06 due to irregularity of temperature and rainfalls during early 2007; thus, it is not done.

4 DISCUSSION

Heterosis-relevant QTL were detected for numerous traits in field and greenhouse trials. By combining data from the DH and BC populations, classes of QTLs were determined based on their effects. How strongly each QTL affected a particular trait was important in associating patterns of phenotypic level with the QTL that were detected. One may assume, for instance, that two *genetically* correlated traits, as estimated in this study, can be traced back to the same QTL. Alternatively, a QTL for a trait may affect another QTL responsible for another trait, which may lead to a causal relationship. However, if the contribution of a QTL to the trait variation is weak, an attempt to relate QTL to a quantitatively variable phenotype becomes challenging.

The following discussion begins with an interpretation of the phenotypic patterns shown in the respective greenhouse and field trials, followed by an interpretation of detected QTL with a focus on QTL related to heterosis-relevant effects (dominance and dominance-derived epistasis). Finally an attempt will be made to relate the phenotypic patterns related to heterosis to the QTL composition. The results will be discussed in relation to other relevant work and with an outlook towards further investigations of the genetic control of heterosis.

4.1 Phenotype patterns

4.1.1 Heterotic patterns

Comparing the DH and BC data distributions provided evidence for the existence of heterosis. Slight differences were seen in the peaks from the two population distributions for almost all the traits observed, in greenhouse as well as in field. This indicated changes in performance due to the genetic structure of the respective populations. DH lines contain only homozygous loci, whereas half of the BC population members are expected to be heterozygous for any given locus, whereby the degree of heterozygosity of each of its members depends on different alleles with ‘Express 617’. Considering the genetic structure, the BC populations has the ‘advantage’ of heterozygosity which is related to heterosis. This probably accounts for the general performance increase in the BC population compared to the DH population.

From a quantitative genetics perspective, a population that consists of heterozygous individuals (e.g. BC test hybrids), has an additional driving effect due to dominance and dominance-related epistasis. The presence of these effects alters the additive contribution from each allele due to the interaction between them (additive-to-additive interaction). Although a negative effect is possible, in general heterozygosity brings an overall positive improvement to the phenotypic performance. This is why heterosis and hybrid vigor are often used as interchangeable terms. The results obtained from the present study simply support this expectations, since there were more positive improvements from the DH lines to the respective BC test hybrids.

Another common feature seen in the phenotypic observations of the DH and BC populations is that variability was generally higher among the DH lines than among the BC hybrids. This was the case for almost all of the traits observed in both the greenhouse experiment and the field trials. Such relative performance is expected, simply because the BC hybrids are obtained by backcrossing each DH line with 'MSL-Express'. Thus each member of the BC population shares half of its alleles, meaning that the allelic pool of the BC population has lower variability than that of the DH population.

The field trials over two growing seasons made it possible to compare the performance of the materials between 2005/06 and 2006/07. The DH population performed relatively consistently for plant height, yield and TSM (despite a clearly lower yield and plant height in 2006/07 in comparison to 2005/06), whereas the BC population tended to cluster resulting in a lack of correlation patterns. Mid-parent heterosis had different patterns for each of the traits reported here. Plant height, which was measured at the end of flowering season, had positive heterosis recorded for both years in almost all accession pairs involved in the trial, although an apparent lack of consistency was shown by many of the accession pairs since a low correlation existed between the two years of observations. Yield, on the other hand, showed a clear difference in pattern between 2005/06 and 2006/07. There was clearly more positive mid-parent heterosis in 2006/07 than 2005/06. In 2005/06, roughly half of the DH-BC pairs showed negative MPH. On the other hand, the parental line 'Express 617' performed poorly in 2006/07, leading to a reduction of mid-parent values and hence a higher estimate of mid-parent heterosis.

4.1.2 Correlations among traits

One aim of this study was to search for possible relationships between early morphological traits with traits in the later stage of development, and particularly with the seed yield. Such information could help us to learn about certain physiological or developmental advantages that are maintained from an early stage throughout the plant's lifecycle. Practically, this knowledge may help us to identify gene loci influencing early development that are critical for improved seed yield.

The trait correlation patterns within the mid-parent heterosis dataset did not meet the expectations. There were significant correlations of certain early morphological traits to plant height and yield (Figure 3.14). Shoot dry weight showed a low but signficant correlation to yield in both 2005/06 and 2006/07. Another early trait, specific leaf weight, showed a significant but low correlation to plant height in 2005/06 and yield in 2006/07. Although low, these correlations indicated that certain relationships among traits existed in the heterotic expression at a morphological level. The DH population showed slightly higher correlations of fresh and dry shoot weight with yield and plant height from both harvest years. These indicated that *per se* biomass building activity, which estimates additive action, influences different traits separated in time. Thus, we have clear indications for correlations between early and later developmental traits, and these correlations are influenced by both additive and dominance actions.

Quijada et al. (2006) observed correlations among field traits from DH lines and their respective test crosses and concluded that the correlation pattern between traits in the DH and testcross populations were different. Exceptions were between plant height and day to flowering, as well as between seed yield and test weight. Unfortunately, that study provided no information relating early development traits with traits at later development or harvest. In the study of Quijada (2006) there was an indication that yield and plant height had a close additive correlation, based on DH data set analysis, however seed weight was correlated with neither of those traits.

Comparing scatter plots from the 2005/06 and 2006/07 field trials in the present study revealed that heterosis expression in the BC test hybrids was not stable in different environments relative to the performance of the DH lines. That did not mean, however,

that the BC test hybrids had a lower performance. The hybrids still showed higher average trait values than the lines, however the range of trait values was smaller than that of the lines. In other words the performance of the poorest DH lines was compensated by hybridisation, whereas the performance of the best DH lines was not improved so dramatically. This suggests a role of dominance effects that were already expressed in the best DH lines because positive alleles from the recurrent parent 'Express 617' were already present.

4.2 Genetic mapping

The development of the genetic map used for the QTL analysis was a stepwise procedure. In the beginning 855 markers were involved. Excluding markers with significant deviation from the expected 1:1 segregation resulted in a preliminary genetic map with 694 markers in 22 linkage groups. Three linkage groups were excluded since they were too short or contained only two or three markers. The subsequent removal of co-localising markers from the remaining groups left 519 markers in 19 linkage groups. Throughout the process remapping was not applied, since reduction of markers may lead to rearrangement of marker positions and result in confusion when comparing the full version of the map with the reduced version.

Allocation of linkage groups to specific chromosomes was done by comparing the map with a reference map published by Piquemal et al. (2005), an aligned map from the DH population 'Express 617' × 'R53' (Radoev 2008), and with map positions provided by SaatenUnion Resistenzabor GmbH for a further set of commercial SSR markers. Development of the genetic map used in this study and the 'Express 617' × 'R53' map was synchronized by applying a number of common SSR markers. A total of 41 SSR markers and two AFLP markers were used to align 16 linkage groups. Final verification was achieved by comparison of chromosome linkage group lengths and orientations with a dense commercial SSR map (information from Jörg Schondelmaier, SaatenUnion Resistenzlabor GmbH). As the result, some of the markers were excluded in the final map, leaving 475 markers in 19 linkage groups representing the 19 *B. napus* chromosomes. The map was published in Basunanda et al. (2007), while the mapping population and mapping data are available as a public resource as part of the Multinational *Brassica* Genome Project (see http://www.brassica.info/resources.php).

4.3 Quantitative trait loci

4.3.1 Genetic action of heterotic QTL

Comparison of the BC and DH data sets gave useful information on the genetic action of QTL with respect to heterosis, whereas epistasis actions on heterosis could be determined from the heterosis data set. Epistatic heterotic QTLs were found throughout all linkage groups except N04, although N04 did contain epistatic QTLs detected using the BC data set. Pooling all contributions of heterosis-relevant epistatic QTL according to trait, and comparing them with their main-effect counterparts, revealed their significant and strong contribution. Some of the epistatic heterotic QTLs had an even higher contribution to the phenotypic variation than some main-effect heterotic QTLs.

It should be mentioned, however, that the estimates for epistatic effects were linear functions of all epistatic component effects. Nevertheless, these results gave clues about the critical role of epistasis in controlling heterosis. By applying genetic marker data, Yu et al. (1997) reported that epistasis between loci played a role in rice yield. Subsequent results in rice also confirmed this (Luo et al. 2001, Hua et al. 2003). In oilseed rape, Radoev et al. (2008) reported that epistasis was strongly involved in heterosis. Before molecular markers covering the entire genome were available, detecting epistasis effects relevant to heterosis was problematic due to overparameterisation of the available mathematical models (because not enough generations were available to fulfil the need for proper statistical analysis) or because simpler models were insufficient for interaction analyses.

The dominance effects that could be estimated using the available data sets enabled a description of the type of dominance that played a role in the respective heterotic main-effect QTL. There were no such QTL detected for early morphological traits from the greenhouse trial, but field trials provided some QTL showing dominance. Partial dominance invariably detected for all the QTL (Table 3.9). This corresponds with previous studies which showed, as marker-assisted studies of QTL genetic action became possible, that many QTL showed some degree of semidominant or partially dominant behaviour (Tanksley, 1993). Moreover, most of the QTL showed dominance toward ‘Express’ parent, which showed large agreement with the phenotypic data obtained from

2007 harvest, as well as the hybrid, that was in favor of 'Express' parent, although due to weather anomaly 'Express' was underperformed by 'V8', a condition that was not expected since 'V8' was taken as parent due to its low GCA (Spiller 2006).

Results from harvest in 2006 unfortunately could not be verified since neither the parental line 'V8' nor its hybrid with 'MSL Express' were tested in the field in that year. Partial dominance shown by two QTL for TSM in 2006/07 (in N07 and N10) was in favor of 'Express 617'. They were relatively weak, compared to that of the other one for 'V8'. To explain that the F1-hybrid showed a similar TSM to 'Express 617', other factor was needed than just additive and dominance.

The main-effect heterotic QTL, in comparison to epistatic QTL, did not have uniform patterns with regard to the traits observed. For thousand seed mass (TSM) from both harvest years, the contribution of main-effect QTL were more or less equal to the contribution of epistatic QTL. For plant height in 2006/07, no epistatic QTL for heterosis were found for field traits, but there was a greater contribution from epistatic QTL than from main-effect QTL for plant height in 2005/06. Yield was dominated by epistatic QTL in both years.

The absence of main-effect QTLs detected for early morphological traits is unexpected since, phenotypically, there were significant differences for the traits observed. However, in similar work done by Radoev (2008), using DH and BC populations derived from cross of 'Express 617' with the resynthesised rapeseed line 'R53', it was found out that there was only one main-effect heterotic QTL for fresh shoot weight at 28 days after sowing. However this QTL on chromosome N03 is potentially co-located with a heterotic main-effect QTL for yield in the 'Express 617' × 'V8' population. Main-effect heterotic QTLs for field traits were also observed in the 'Express 617' × 'R53' populations, and indications were also seen for co-location of heterotic QTLs between the two populations (Basunanda et al., 2009).

4.3.2 Possible loci involved in plant height and thousand seed mass (TSM)

Heterotic QTL for plant height and TSM raise the question of what genes might be represented by these loci. Both are related to traits that have been studied more

thoroughly in numerous model and crop species. In a study of flowering time, for example, Thornsberry et al. (2001) found an influence of the gene *Dwarf8*. This gene is known to be the member of *DELLA* protein gene family, whose orthologs including *GAI* (GA insensitive) were found in *Arabidopsis thaliana* to control height (Peng et al. 1999; Hussain & Peng 2003). Orthologs involved in plant height have also been identified in wheat (as *Rht*), and a less similar ortholog (*Vvgai*) with an analogous effect on GA signaling was also found in grapevine (Hussain & Peng 2003). In *A. thaliana*, Johanson et al. (2000) found that the vernalisation and flowering-time regulator *FRIGIDA* (*FRI*) also plays a role in development, while Caicedo et al. (2004) found that epistatic interactions between *FRI* and the flowering time regulator *FLC* can have a general effect on development. All of the genes mentioned are transcription factors, not coding enzymes. Although *FRI* and *FLC* are not from GA pathway, but involve in the vernalisation cascade, they are known to also influence hormonal pathways within the plant and therefore presumably have more global effects.

QTL hotspots for heterosis in biomass traits and metabolites, respectively, were reported recently in Arabidopsis by Meyer et al. (2009) and Lisec et al. (2009). Significant clusters of heterotic metabolite QTL were observed by Lisec et al. (2009) at the bottom of Arabidopsis chromosome 1, the bottom of chromosome 3 and the top of chromosome 4. The latter two regions coincided with significant clusters of *per se* and heterotic biomass QTL in the study of Meyer et al. (2009), while the cluster on chromosome 4 also contained multiple *per se* metabolite QTL (Lisec et al. 2008) and was also involved in digenic epistatic interactions (Meyer et al. 2009). Both regions are known to contain numerous flowering-time related genes, and *FRI* is one of the potential regulatory candidate genes identified by Meyer et al. (2009) in the main heterotic QTL cluster at the top of Arabidopsis chromosome 4.

Other possible gene candidates related to plant height are *Brrga1*, found in *Brassica rapa* (Muangprom et al. 2005), and *BREIZH* (*bzh*) (Foisset et al. 1995) as well as *ndf1* from *Brassica napus* (Wang et al. 2004). *Brrga1* is, again, a member of *DELLA* protein gene-family and has non-lethal, dwarfing effect in *A. thaliana* and *B. napus* when transformed (Muangprom et al. 2005), however allelic variants may have non-dwarfing phenotypes that influence hormone biosynthesis and gene regulation.

Seed size (TSM) has been intensively studies in Arabidopsis. Garcia et al. (2005) proposed a cross-talk model between genes in zygotic (endosperm) and maternal (integument) tissues as the controlling mechanism that leads to the final potential size of seeds. *TTG2* (*AtWRKY44*, *At2g37260*) and *KRP2* genes in the integument are involved in cell elongation and division. These genes work independently to the *HAIKU* (*IKU*) class of genes that is expressed exclusively in the endosperm. However, there is interaction between both mechanisms which determine the seed size.

Luo et al. (2005) elaborated on the way in which two *HAIKU* genes, *IKU1* and *IKU2* (*At3g19700*), could be related to the *MINI3* (*AtWRKY10, At1g55600*) gene in regulating endosperm (seed) size. They suggested that the genes are involved in a single pathway, with *IKU1* located furthest upstream followed by *MINI3* and *IKU2*, consecutively. It is interesting that *TTG2* and *MINI3* both belong to the same gene *WRKY* family of transcription factor genes, which has 74 members in Arabidopsis (Ülker and Somssich 2004). *IKU2* is a member of LRR receptor kinase family of genes involved in cell signaling. Recent findings uncovered a possible role of the photosensitive gene *SHB1* expressed in the embryo (Zhou et al. 2009) and its epigenetic influence in form of DNA-methylation controlled by expression of *MET1* in somatic and flowal gametic cells (Fitzgerald et al. 2008). The latter was suggested to cause a significant maternal effect on final seed size.

4.3.3 Nature of heterotic QTL

The results obtained in the present study show that the detection of heterosis-relevant QTL depended on the trait observed, but n all cases was environmentally influenced. This was clear from different QTL that were found in different years or types of trial. Although there were some co-locating QTL from the same trait in different years, or from different traits or trials, many of the QTL found were located individually. On the other hand when clusters of QTL (active regions) for different traits, trials or years were considered, the co-localisation gave another meaning. QTL observed in active regions might have a common physiological or biochemical basis impacting the traits involved.

Regulatory genes are known to affect multiple genes, either by regulating different genes (common regulators) or by regulating a key gene which in turn influences other genes in

a subsequent cascade. The bulk of QTL are known to be regulatory (Birchler et al. 2001). As will be discussed below, such QTL belong to the class of polygenes and are subject to minor mutations that occur throughout the cell lifecycle. Dynamics of environmental influences force selection to these mutation products as a mean to maintain variation (Barton and Keightley 2002).

Many epistatic interactions were detected contributing to heterosis in this study. In fact, the number of main-effect heterotic QTL detected was less than the number of epistatic heterotic QTL (Table 4.1). While epistasis has been known to affect heterosis, most studies avoid elaborating it further. This is not surprising, since epistatic interaction is often viewed as "complicating factor", especially if one tries to exploit it in selection. Hence the results of this study will be very difficult to use for practical breeding. More than 90% of the interactive QTL detected are QTL only give significant effects in the interacting state (see Table 3.2 and Table 3.4). Only a small fraction are Type II interactive QTL, while no main-effect-QTL to main-effect-QTL (Type I) interactions were detectable. Even if we consider active regions as QTL, heterosis for complex traits seems to result from highly complex activities of many genetic factors. Furthermore, as with main-effect QTL, epistatic QTL may be pleiotropic and thus involved in more than single trait by influencing common physiological or biological pathways.

A weak influence of the QTL on the observed phenotypic variation, as approximated by their "heritabilities", indicated that applying only QTL analysis is not sufficient to study heterosis on the molecular level. Critic to this type of analysis (QTL analysis) is that it applies a (statistical) level of significance in deciding which loci to elaborate further. While this threshold is important to dissect "true" QTL from random disturbances, evidence that small contributing QTL are involved (maybe in a constant interactive state) makes it likely that this concentration on statistically significant QTL causes a loss of information.

The understanding of heterosis or hybrid vigor is developing interestingly with the incorporation of more sophisticated molecular tools. By the end of the 20th century, the debate on dominance versus overdominance theory was still not decisively solved, even with the help of molecular techniques such as QTL analysis. As mentioned in the Introduction, some studies found that hybrid vigor was more influenced by dominance,

Table 4.1 Number and percentage contribution of main-effect and interactive (epistatic) heterotic QTL detected in greenhouse and field trials of 250 DH lines from the cross 'Express 617' × 'V8' and their respective BC test hybrids with 'MSL-Express'.

Trait	No. of main-effect QTL	No. of epistatic QTL pairs	Total contribution of main-effect QTL (%)	Total contribution of epistatic QTL (%)
Greenhouse trial				
Shoot dry weight	0	1	0	6
Shoot fresh weight	0	5	0	34
Leaf dry weight	0	2	0	12
Leaf fresh weight	0	4	0	27
Hypocotyl length	0	2	0	13
Leaf area	0	4	0	20
Specific leaf weight	0	1	0	5
Field trial				
Yield 2006	2	3	8	17
TSM 2006	1	2	12	12
Plant height 2006	1	2	5	8
Yield 2007	2	8	7	30
TSM 2007	3	3	14	16
Plant height 2007	2	6	6	32

while others concluded that overdominance or pseudo-overdominance was involved more. However, epistasis has also been implicated in many cases, and evidence from more detailed molecular studies is providing more and more support for this cause (e.g. Lisec et al. 2009, Meyer et al. 2009). Although clear results were obtained from the model plants rice and Arabidopsis, with small and fully-sequenced genomes, results from oilseed rape or other *Brassica*s are more difficult to interpret due to their larger, less well characterised genomes and plastic phenotype nature caused by genome duplications and polyploidy. This study reinforces the need for more detailed analysis of the heterosis phenomenon in *B. napus*, for example using genome-wide SNP maps.

The integration of quantitative genetics concepts, such as applying estimations of additive, dominance or epistatic effects to QTL studies, may help to understand the mechanisms behind heterosis but apparently is not adequate for complete understanding. Quantitative genetics estimation methods are based on the assumption that genes related to a trait each have a small contribution and their effects sum up additively to build the trait. The effects are dissected into three major groupings – additive genetic, dominance, and epistatic effects – which estimation is purely statistical. While for a complex trait this assumption is acceptable (since we do not know exactly how it develops from many genes), application of molecular techniques gives hints that in gene expression level the interplay of factors affecting expression of genes is considerably more complex.

Birchler et al. (2007) suggested the role of dosage-effects on heterosis at the expression level. The theory he and others propose is not directly linked to heterosis, rather it basically supports the idea of "multiple genes", the basic tenet of quantitative genetic principles (Birchler et al. 2001). Instead of being "summed up additively" like in classical quantitative genetics, however, any target gene is subject to certain hierarchical regulations. A target gene is regulated by transcription factors, and these in turn are also modulated by modifier genes which mostly act additively, implying dosage-effects. The theory does not exclude epistasis, since transcription factors usually react negatively to dosage-effects, causing a compensation process. An increase in number of genes controlling a certain trait may not affect the increase in level of expression if their respective transcription factors react negatively. The study of Semel et al. (2006), which supports the existence of overdominant QTL and rejects that the action is simply pseudo–overdominance, raised the possibility of dosage-effect mechanisms controlling heterosis.

It is widely known that many QTL are members of signal transduction cascades or are transcription factors. These two classes of genes have a known tendency to exhibit dosage-modifying effects (Birchler et al. 2001).

In a broader perspective, heterosis is increasingly evident as part of evolutionary processes. The results of Semel et al. (2006), using a set of test-crossed near-isogenic inbred lines from an interspecific cross of diploid tomatoes, revealed that overdominant actions were detected only for reproductively related traits, while this mode of action was absent for other traits. Each line was characterised with respect to its regional genome differences from one of its parents, to ensure a minimal possibility for epistasis to occur. Based on this study, heterosis was seen by Semel et al. (2006) as a strategy to survive or to maintain the gene-pool.

If heterosis is really part of the (molecular) evolutionary process, it is not impossible that various molecular evolutionary mechanisms may shed light on our understanding oh how heterosis develops. One explanation on how genes evolve is through modification after mutation. One mutation process often associated with modification is gene duplication. As described by Louis (2007), the fate of duplicated genes can be (1) loss of function (the most common) in one of the copies, (2) divergence of functions in both copies, (3) complementary of functions, or (4) differential regulation due to modifications in regulatory regions. A common cause of such modifications is gene methylation, a widespread epigenetic mechanism.

Using *Saccharomyces cerevisiae*, Hittinger and Carroll (2007) explained the fate of an apparently bifunctional ancestral gene which experienced duplication followed by complementary functionality and adjustment of each regulatory region. These genes, now known as *GAL1* and *GAL3*, are paralogs involved in the same biochemical pathway, but have different functions. *GAL3* is the co-inducer of *GAL1* activity, by sequestering the repressor for transcription factor to activate *GAL1*. *GAL1* is known to have a multiplied activity compared to its ancestral type, which can still be found in *Kluyveromyces lactis*, both by “dividing the tasks” and by making the regulation more effective. By realising that duplication may lead to complementation or differential regulation, one may deduce that such evolutionary mechanism can result in development of heterosis.

Recent findings in maize apparently support the theory of hemizygous complementation (Hochholdinger & Hoecker 2007). Inbreds of maize were known to have disrupted gene collinearity; that is, loss of particular copies of genes. In hybrids this loss is partly compensated, leading to more effective biochemical activities and, in turn, better performance. Inbreeding will disrupt the compensatory effect in some inbreds, thus one may observe inbreeding depression.

4.4 Role of expression studies

Expression studies provide a possibility to connect QTL with expression data. Whereas expression of a small number of genes can be studied using low-throughput gene expression techniques, such as real-time PCR, it is now possible to conduct whole-genome studies using high-throughput expression techniques. Beginning with microarray technologies, innovative methods have since been developed that can simultaneously reduce the costs and increase the efficiency of global transcriptome profiling. In particular, a combination of Serial Analysis of Gene Expression (SAGE) (Velculescu et al. 1995, Obermeier et al. 2009) with new sequencing technologies gives the opportunity for ultradeep transcriptome profiling. The newest DNA sequencing methods, together dubbed “massively parallel” or “next-generation” sequencing, can generate millions of sequence tags from a series of transcriptome libraries within a few days (Ju et al. 2006, Shendure et al. 2005, Gnirke et al. 2009). In contrast to microarray analysis, next-generation sequencing in combination with SuperSAGE (Matsumura et al. 2003) can also enable exact quantification of differential expression of rare transcripts or unknown genes.

Abundant information of differential expression data in segregating populations will make it possible to relate this with trait and genetic marker data. By mapping expression profiles onto a genetic map it is possible to obtain expression-relevant positions in the genome using a technique known as expression QTL (eQTL; Gibson and Weir 2005). This approach, dubbed genetical genomics (Jansen and Nap 2001), enables one to determine which QTL behave as *cis*-acting genes and which as *trans*-acting loci with a regulatory effect on more complex networks. Although such techniques are still relatively expensive, they have good potential for identification of transcription factors underlying complex traits. Nettleton and Wang (2006) described techniques for the use of selective

transcriptional profiling for trait-based eQTL mapping. This approach, in which groups of extreme phenotypes that show marker segregation at major QTL are selected for the differential expression analysis, can enable a reliable localisation of trait-related eQTL in a relatively small subset of mapping genotypes; therefore the costs of effective eQTL analysis can be considerably reduced. Applying the techniques with our data will unveil more active factors controlling heterosis in the trait studied. Recognizing *cis*-acting or *trans*-acting QTL and identifying the underlying genes will potentially give a wealth of new information on the nature of heterosis and related the epistatic interactions involved in expression of heterosis effects.

5 SUMMARY

Development of hybrid cultivars increasingly becomes part of strategies to achieve higher oilseed rape production. In Germany, hybrid cultivars dominate the oilseed rape seeds market, despite high concurrence from line cultivars, the more traditional type. Involving molecular marker-assisted technologies may potentially reduce production time and cost of hybrid varieties, which is known to be time consuming and resource demanding. Markers are used to detect quantitative trait loci (QTL) that are responsible to the trait in question. Since the central theme in hybrid breeding is heterosis – a genetic phenomenon displayed by heterozygots, in which they show better performance than their parents – displayed by the traits of interest, it is important to put the first emphasize on the QTL relevant to answer heterosis. The genetic action of the QTL found furthermore is necessary to be studied, as this will become the basis for decision on which breeding method to be used.

In order to study this, a mapping population of a series of doubled-haploid (DH) lines from a cross between 'Express 617' and 'V8' was developed from an F1 plant using microspore culture. The population was used to develop genetic map and involved in QTL analysis. A counterpart series of back-cross hybrid (BC) population was also developed in 2004/05 by crossing each line in DH line population with the 'Express 617' parent. The mapping populations would provide estimates of genetic effects. By using information provided by both populations, estimates of genetic effects of QTL, including the heterosis-related ones, could be obtained.

Phenotypic performance was studied through two-stages study: in greenhouse and in field. Greenhouse trial was conducted from May to August 2007 to provide information on early development stage of growth. Field trial was conducted in four locations, namely Einbeck and Reinshof in Lower Saxony as well as Rauischholzhausen and Grund-Schwalheim in Hesse; all were evaluated for two planting seasons (2005/06 and 2006/07). To handle the great number of accessions, an alpha lattice design was utilized with 26 blocks of 26 plots each and locations were treated as replicates. Plant height at the end of flowering time, seed yield, and thousand seed mass were observed.

Microsatellites (SSR) and AFLP® were used to develop genetic maps using the mapping population. The genotyping was done using Licor machine involving 527 AFLP® and 176 SSR markers toward 250 genotypes in order to built a genetic map. The genetic map, generated using JoinMap® 3.0, comprised all the chromosomes of *Brassica napus* (Chromosome N01-N19) covering 1792 cM. From this, 269 markers – 144 of which are SSR markers – were chosen as backbone map in the subsequent QTL analysis. Three sets of data were analysed for each trait, namely from DH-lines population, from BC population, and from mid-parent heterosis values that were calculated from the difference between BC values and average of their respective parents.

Greenhouse trial showed that BC population tended to perform slightly better than DH in almost all traits observed. Its average value was higher and it had a smaller range distribution than DH. Heterosis distributions were varied among traits, which range spanning from -31% (leaf area) to 84% (shoot dry mass). The proportion of the crossing pairs showing positive heterosis was higher than the ones showing negative one over all the traits observed, which was typical. The correlation coefficients between BC and DH population among the traits observed were medium, but in hypocotyl length it was high (0.81) and in specific leaf weight was very low. Correlations among traits were high between shoot weight and leaf area in DH and BC population; however, in MP data set they were reduced, indicating that heterosis were developed independently from their parents for each pair.

Three traits were observed in the field trials that took place for two planting years (2005/06 and 2006/07): seed yield (yield), thousand seed mass (TSM), and plant height at the end of flowering. Seed yield for 2005/06 was clearly higher than the next planting year. Mild winter and dry period during flowering time by 2006/07 caused the yield to go down 30% in average. DH was less affected by year effect than BC; however BC showed less inner-variation than DH. The data distribution showed that BC had tendency to higher value than DH for both planting years. Mid-parent heterosis in 2006/07 was better (higher) than the previous year.

Thousand seed mass showed different pattern than yield, indicating that it was controlled through different mechanism. DH showed a strong correlation over both planting years, whereas BC and MPH were much weaker. This indicated the inert character of lines.

Strong correlations between BC and MPH indicated that MPH was controlled more by variation in BC. In this trait negative heterosis was abundant and even in 2006/07 planting year more than half of the accession pairs showed negative heterosis. Even the parents and their F1 showed such phenomenon.

When the greenhouse trial results were compared with the field trial ones, it was evidence that MPH correlation between the traits observed in both experiments were invariably low; most of them were insignificant statistically. Of the small number of significant correlations are shoot dry weight with yield from both planting years, specific leaf weight with plant height at 2005/06 and yield at 2006/07. Thousand seed mass appeared to be unconnected, as well as hypocotyl length and leaf area. Biomass (fresh shoot mass) in general might show a weak correlation with yield.

From greenhouse trial there was no heterosis-relevant main-effect QTL detected. Eleven main-effect QTL nevertheless was found from DH and BC populations; however, they were not co-located, making it impossible to indirectly estimate dominance effect.

In opposite to greenhouse trial, there were eleven heterosis-relevant main-effect QTL detected in field trials: four from 2005/06 planting year and seven from 2006/07. All the traits observed from both planting years were represented. As already indicated from phenotypic relationship, QTL for thousand seed mass were mostly different from the other traits. Chromosome N07 and N16 were especially unique for the heterosis-relevant QTL for thousand seed mass although N16 also contained heterosis QTL for yield. Heterotic QTL for yield were detected at N03, N08, and N13, beside N16. Heterotic QTL for plant height located at chromosome N03, N05, and N11.

Since there were overlaps among QTL and the distribution of QTL was not random, clusters of main-effect QTL were called ‘active regions’ and each was seen as a single region, ignoring the traits. Twenty six active regions were detected on 15 of the 19 chromosomes. Only N02, N04, N15, and N18 did not have active regions, although they did have epistatic QTL, of which some interacted with QTL located in active regions. Chromosome N09 had the most active regions, namely five. Certain active regions contain QTL that control wide range of traits, such as the one in chromosome N05. This active region, however, is very long, almost 50 cM. Most single trait active regions were

due to thousand seed mass, indicating a much different control of this trait from the others, although in certain regions there were also overlapping with other trait, as in chromosome N09 with yield 2005/06. Certain interacting QTL were also found connecting different active regions, which may also indicate possible co-regulatory function.

A notable feature of the detectable epistatic interactions was that Class I interactions, i.e. interactions between two main-effect QTL, were non-existent in both the field and greenhouse trials. Purely interactive QTL dominated the epistasis cases. Applying "active region" as basis for interaction unit raises the percentage of interaction involving at least one active region from 5% to 65%. This suggested that, at least in case of epistasis, it is better to see a cluster of QTL as single unit. Moreover, certain interactions involving the same trait could be found clustering in the same location. Such feature is quite interesting, since it may indicate existence of transcription factors.

QTL effects related to heterosis from early traits (greenhouse trials) were evidently dominated by epistatic origin, since no dominant QTL was detected directly from MP data set. Nevertheless, four main-effect QTL were found from BC data set, estimating difference between genetic additive and dominance effect; two of them, responsible for dry shoot weight and fresh shoot weight, located at the same region in N14.

Observation on traits from field trials provided us with estimates of dominance obtained from main-effect QTL either found in MP or BC data set. Heterosis-related epistatic effects were also abundant covering all traits. Certain main-effect QTL co-located with epistatic QTL, either from the same or different traits, lead to possible pleiotropism, i.e. QTL or a trait affecting other trait(s).

Further investigation on the type of dominance of a heterosis-relevant main-effect QTL, that is whether they showed partial, complete dominance, or overdominance is not always easy since most of main-effect QTL showing heterosis did not co-locate at the same position with other main-effect QTL from the same trait that had additive effect (from DH data set). Nevertheless, partial dominance played in all the QTL for heterosis which additive and dominance effects could be estimated. Five of the seven such QTL showed partial dominance toward 'Express', the other two toward 'V8'. Most of the estimates

were from QTL for thousand seed weight The other two were for plant height and seed yield in planting year 2005/06.

The results showed that epistasis was evidently the source of heterosis in the early developmental stage. Dominance was found to play role in the traits observed at end stages of life, although epistasis was still abundant; most of the dominance were partially dominance, with one (for yield) showed overdominance. Phenotypic correlation between shoot dry weight at early stage and yield could not be strongly supported by the QTL analysis. Genetic analysis involving expression of gene, such as e-QTL can be used to explain the mechanism behind the correlation, which further can be applied in developing more effective marker-assisted methods to detect heterosis-relevant alleles.

6 ZUSAMMENFASSUNG

Die Entwicklung von Hybrid-Sorten findet zunehmend Anwendung, um die Rapsproduktion zu steigern. In Deutschland dominieren heute die Hybridssorten den Saatgutmarkt von Raps trotz starker Konkurrenz durch die traditionellen Linien-Sorten. Die Nutzung molekularer Marker, um quantitative Merkmale Loci (QTL) zu lokalisieren, eröffnet die Möglichkeit, gewisse Nachteile der Hybridzüchtung - wie hohe Kosten und großen Zeitaufwand - zu reduzieren. Der Hybridzüchtung liegt das Phänomen der Heterosis zugrunde, das eine höhere Leistungsfähigkeit von Heterozygoten gegenüber ihren Eltern bezeichnet. Daher erlangen Heterosis-relevante Gene bzw. QTL eine zunehmende Bedeutung. Dabei ist es auch notwendig, die genetischen Effekte der gefundenen QTL zu untersuchen, da auf der Basis dieses Wissens Entscheidungen zur Wahl der geeigneten Züchtungsmethode getroffen werden können.

Für die vorliegende Studie wurde über Mikrosporenkultur eine Doppelhaploid-Population (DH) aus der Kreuzung 'Express 617' × 'V8' (F1) entwickelt. Die Population wurde verwendet, um eine genetische Karte zu entwickeln und QTL zu berechnen. Darüberhinaus wurde in der Vegetationsperiode 2004/05 eine Rückkreuzungspopulation (Backcross, BC) aus Kreuzungen jeder DH-Linie mit dem Elter 'Express 617' entwickelt, um QTL-Analysen anhand dieser Population und eine Bestimmung der Heterosis (*Mid-parent heterosis*) zu ermöglichen. Auf der Grundlage der ermittelten genetischen Effekte der QTL kann die Ursache des Heterosis-Effekts bestimmt werden.

Von Mai bis August 2007 wurde ein Gewächshausversuch durchgeführt, um Informationen über die frühen Wachstumsphasen der Rapspflanzen zu gewinnen: gemessen wurden die Blattfläche und die Biomasseakkumulation. In den Jahren 2005/06 und 2006/07 wurden Feldversuche an vier Standorten, Einbeck und Reinshof (Niedersachsen) sowie Rauischholzhausen und Grund-Schwalheim (Hessen) durchgeführt; an allen Prüfgliedern wurden in beiden Vegetationsperioden die Pflanzenhöhe zum Blühende, der Samenertrag und die Tausendkornmasse (TKM) gemessen.

Als molekulare Marker wurden Mikrosatelliten (SSR) und AFLP® verwendet, um die Linien zu genotypisieren und genetische Karten zu entwickeln. Die genetische Karte wurde mit JoinMap® 3.0 berechnet und umfasste alle 19 Chromosomen (N01 bis N19)

des Genoms von Brassica napus und hatte eine Gesamtlänge von 1792 cM. Für die QTL-Analyse wurde eine Basiskarte mit 269 ausgewählten Markern erstellt. Die Berechnung der QTL wurde mit der Software QTL-Network 2.0 durchgeführt. Die analysierten Daten bestanden aus drei Gruppen von Werten: DH-Linien, Rückkreuzungen (BC) und Heterosis.

Der Gewächshausversuch 2007 zeigte, dass die Leistung der BC-Population in fast allen beobachteten Merkmalen etwas besser war als die der DH-Population. Die Heterosiswerte variierten von -31% (Blattfläche) bis +84% (Sproßtrockenmasse). Außerdem waren die positiven Heterosiswerte häufiger als die negativen. Die Korrelationen zwischen den beobachteten Merkmalen der BC- und DH-Population waren meistens durchschnittlich, wobei die Korrelation bezüglich der Hypokotyllänge jedoch hoch (0,81) und beim spezifischen Blattgewicht (Blattgewicht/Fläche) sehr niedrig war. Die Korrelation zwischen der Sproßmasse und der Blattfläche der DH- und BC-Population war hoch, war aber bzgl. der Heterosis reduziert. Dies ist ein Hinweis darauf, dass die Heterosisleistung meistens von der Leistung der jeweiligen Eltern unabhängig war.

Der Kornertrag im Anbaujahr 2005/06 war deutlich höher als im Folgejahr. Der milde Winter und eine Trockenperiode während der Blüte in 2006/07 haben den Ertrag in Durchschnitt um 30% gesenkt. Dabei war die DH-Population weniger von dem Jahres-effekt betroffen als die BC-Population; trotzdem zeigte die BC-Population selbst aber eine geringere Variation als die DH-Population. Die Verteilung der Ertragsdaten der beiden Anbaujahren ergab, dass die BC-Population höhere Werte aufwies als die DH-Population. Die Heterosis war in 2006/07 ausgeprägter als im Vorjahr.

Die Tausendkornmasse (TKM) zeigte andere Ergebnisse als Kornertrag und Pflanzenhöhe. Dies deutet darauf hin, dass diese Merkmale genetisch unterschiedlich kontrolliert sind. Allerdings zeigte sich für alle Merkmale in der DH-Population eine enge Korrelation zwischen den Anbaujahren, während die BC-Population und die Heterosiswerte schwächere Korrelationen zeigten. Man kann feststellen, dass die Werte der DH-Linien stabiler waren – wenn auch auf niedrigerem Niveau – als die BC-Nachkommen. Das TKM zeigte eine besonders deutliche, negative Heterosis. Selbst im

Anbaujahr 2006/07 zeigten mehr als die Hälfte der Nachkommenschaften eine solche Tendenz.

Die Korrelationen zwischen Heterosiswerten der beobachteten Merkmale aus Gewächshaus- und Feldversuch waren ausnahmslos niedrig und meistens statistisch nicht signifikant. Signifikante Korrelationen konnten jedoch zwischen Sproßtrockenmasse und Kornertrag (in beiden Anbaujahren), spezifischem Blattgewicht und Pflanzenhöhe (2005/06) sowie spezifischem Blattgewicht und Kornertrag in 2006/07 ermittelt werden. Dagegen zeigten TKM, Hypokotyllänge und Blattfläche keine Korrelationen, während die Sproßfrischmasse mit dem Kornertrag schwach korrelierte.

Für die DH- und BC-Populationen wurden im Gewächshausversuch 11 Haupt-QTL ermittelt, die jedoch nicht als direkt heterosis-relevant anzusehen sind. Die QTL kolokalisierten jedoch nicht und schlossen damit die Möglichkeit der indirekten Schätzung von Dominanzeffekten aus.

Im Feldversuch wurden ebenfalls 11 heterosis-relevante Haupt-QTL ermittelt: vier für das Anbaujahr 2005/06 und sieben für 2006/07. Wie von den phänotypischen Korrelationen zu erwarten war, kolokalisierten QTL für Tausendkornmasse nicht mit QTL für die anderen Merkmale. Heterosis-relevante QTL für TKM wurden auf den Chromosomen N07 und N16 lokalisiert, während heterotische QTL für Kornertrag auf den Chromosomen N03, N08, N13 sowie N16 identifiziert wurden. Heterotische QTL für Pflanzenhöhe befinden sich auf den Chromosomen N03, N05, und N11.

Da die berechneten QTL-Bereiche nicht zufällig über das Genom verteilt waren, sondern sich teilweise überschnitten und Cluster bildeten, kann von sogenannten „aktiven Bereichen“ gesprochen werden. Insgesamt 26 solche aktive Bereiche wurden in 15 der 19 Chromosomen nachgewiesen. Nur N02, N04, N15 und N18 zeigten keine aktiven Bereiche, obwohl sie epistatische QTL aufwiesen, von denen einige zu QTL-Effekten in aktiven Bereichen beitrugen.

Chromosom N09 besitzt mit fünf die meisten aktiven Bereiche. Einige aktive Bereiche – wie auf Chromosom N05 – enthielten QTL-Regionen, die ein breites Spektrum an Eigenschaften kontrollieren. Der aktive Bereich ist jedoch mit fast 50 cM sehr groß. Die

meisten aktiven Bereiche mit einem QTL für nur ein Merkmal kontrollierten das Merkmal TKM. Bestimmte epistatische QTL verbanden auch verschiedene aktive Bereiche miteinander, und zeigten so eine mögliche koregulatorische Funktion.

Eine bemerkenswerte Eigenschaft der gefundenen epistatischen Wechselwirkungen war, dass es keine Klasse-I-Wechselwirkungen (zwischen zwei Haupt-QTL) gab. Die epistatischen Effekte waren allerdings dominiert von wechselwirkenden QTL, die keine Haupt-QTL beinhalteten. Verwendet man aktive Bereiche als Basis für die Beurteilung von Wechselwirkungen, erhöht sich der Anteil der Wechselwirkungen, die mindestens einen aktiven Bereich umfassten, von 5% auf 65%. Daraus folgt, dass die Beurteilung von Epistasie zweckmäßiger auf der Basis ganzer aktiver Bereiche, also Haupt-QTL-Clustern erfolgt, anstatt auf Basis von Einzel-QTL. Ferner kolokalisierten bestimmte Wechselwirkungen, die dieselben Merkmale betrafen, was ggf. auf Effekte von Regulatorgenen (Transkriptionsfaktoren) hindeutet.

Die heterosis-relevanten QTL-Effekte von Jugendmerkmalen – berechnet aus den Ergebnissen des Gewächshausversuchs – wurden offenbar von Epistasie geprägt, weil keine dominanten QTL direkt aus den Heterosis-Werten festgestellt werden konnten. Dennoch wurden vier Haupt-QTL in der BC-Population gefunden, die als Schätzungen des genetischen Unterschieds zwischen additiv-genetischen und Dominanz-Effekten gelten können. Zwei von ihnen, verantwortlich für Sproßtrockenmasse und -frischmasse, befanden sich im gleichen Bereich auf N14.

Anhand der Ergebnisse aus den Feldversuchen konnte Dominanzeffekte der Haupt-QTL ermittelt werden. Einige Haupt-QTL, die mit epistatischen QTL kolokalisierten, können möglicherweise Pleiotropie verursachen, d.h. dass ein QTL oder Merkmal andere Merkmale beeinflusst.

Die genauere Charakterisierung des Dominanztyps eines heterosis-relevanten Haupt-QTL, d.h. ob es sich um unvollständige Dominanz, vollständige Dominanz oder Überdominanz handelt, war nicht immer einfach, da die meisten der heterosis-relevanten Haupt-QTL sich nicht an den gleichen Positionen wie die Haupt-QTL mit additiv-genetischem Effekt befanden. Die durchgeführten Schätzungen der heterosis-relevanter QTL zeigten, dass die Effekte unvollständig Dominanz waren. Fünf von den sieben QTL,

deren Additiv- und Dominanzeffekte geschätzt werden konnten, waren ihre Direktionen nach ‘Express‘ geneigt. Die zwei andere waren QTL für Tausendkornmasse, eine von 2005/06 Anbaujahr und die andere von 2006/07 Anbaujahr. Anscheinend, Tausendkornmasse wurde mehr von dem Haupteffekt kontrolliert als die andere Merkmale. Aufgrund nur wenig QTL, die ihre Haupteffekte geschätzt werden konnten, es muss anderen Faktor geben, denen Effekt die phenotypische Expression weiter erklärt.

Die Ergebnisse zeigen, dass Epistasie offenbar eine wichtige Ursache für Heterosis in frühen Entwicklungsstadien der Rapspflanze darstellt. In unseren Untersuchungen spielte Dominanz ein wichtigere Rolle bei Merkmalen, die in späteren Stadien (inkl. Ertragsmerkmale) beobachtet wurden; aber auch hier hatte Epistasie noch einen starken Einfluss. Im wesentlichen wurde unvollständige Dominanz nachgewiesen, im Fall von Ertrag auch Überdominanz.

Die festgestellte phänotypische Korrelation zwischen Sproßtrockenmasse im frühen Stadium und dem Kornertrag konnte durch die QTL-Analyse nicht gestützt oder erklärt werden. Weitere genetische Analysen anhand der Gen-Expression, wie die Erfassung von e-QTL, können zukünftig dazu beitragen, die zugrunde liegenden Mechanismen der Merkmalskorrelation zu untersuchen und die Entwicklung effektiver marker-gestützter Selektionsmethoden auf heterosis-relevante Allele für die Hybridzüchtung zu ermöglichen.

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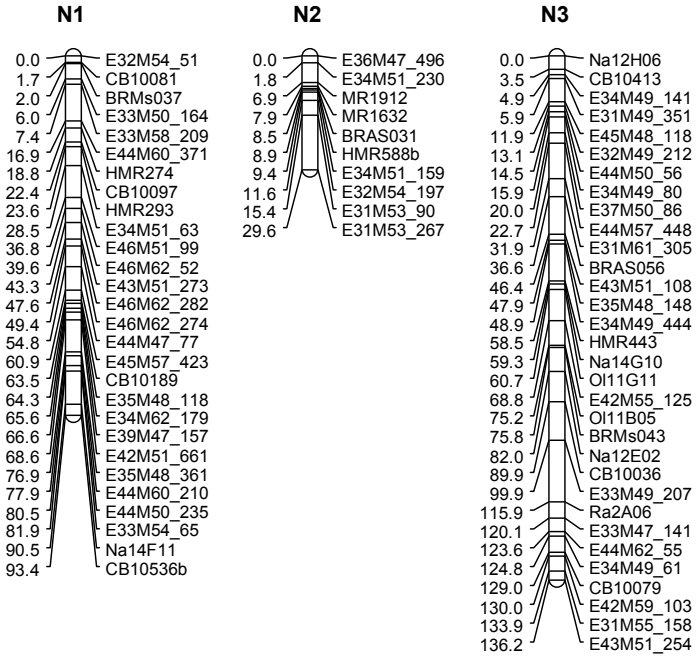
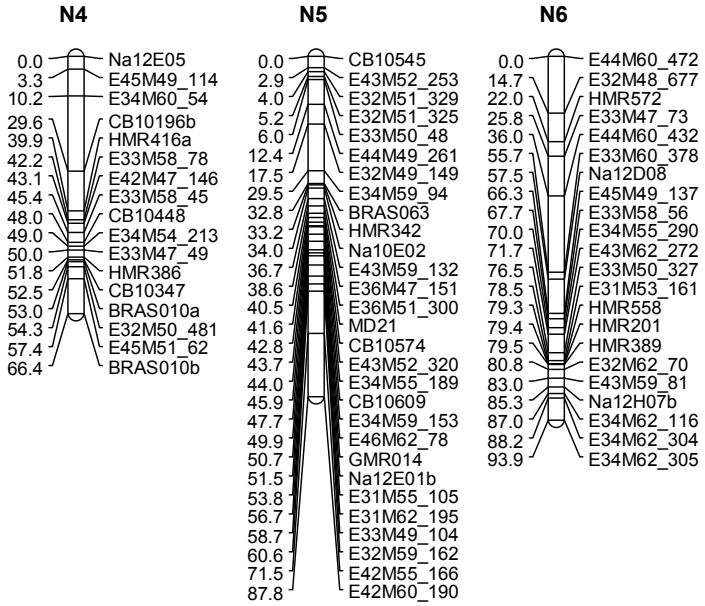
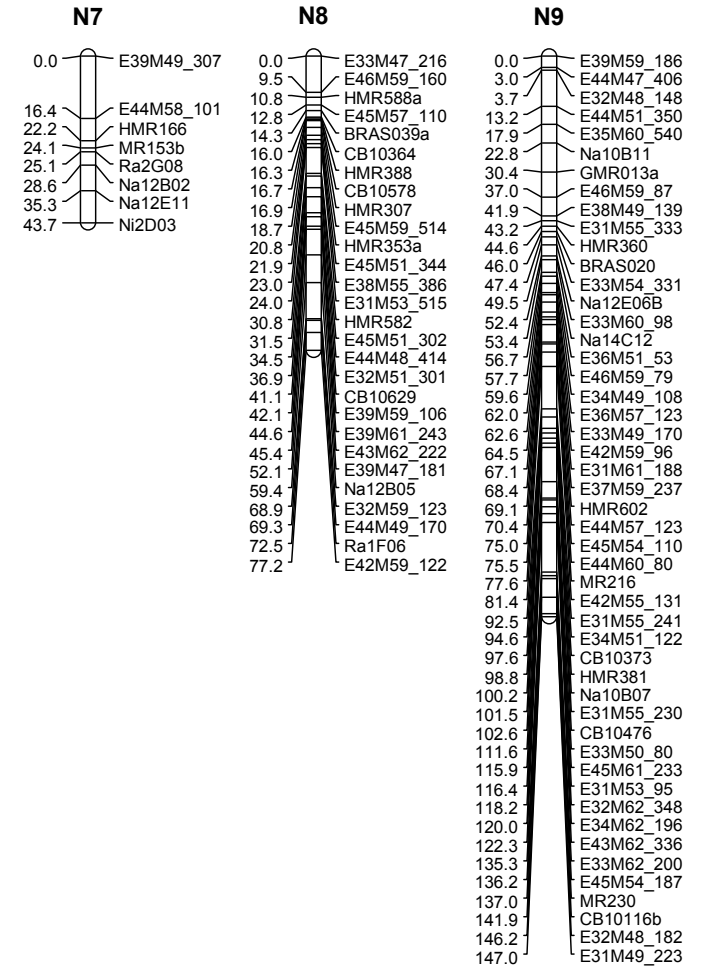
APPENDICES

Appendix A. Genetic map.

Genetic map of the *Brassica napus* doubled haploid (DH) population 'Express 617' × 'V8', containing 419 AFLP and SSR markers grouped using JoinMap® 3.0. SSR markers (with prefices Na, Ni, Ol, and Ra) mainly were already listed at http:// brassica.bbsrc.ac.uk/cgi-bin/ace/searches/browser/BrassicaDB , with additional 32 (from total 35) SSR primer pairs provided by Saaten Union Resistenzlabor GmbH (with prefices HMR or GMR) and 42 (from total 114) primer pairs from the Celera AgGen Brassica Consortium (see Piquemal et al. 2005; with prefices BRAS, CB, and MR). The polymorphic primer pairs were used to genotype 262 DH lines.




N4
0.0 Na12E05
3.3 E45M49_114
10.2 E34M60_54
29.6 CB10196b
39.9 HMR416a
42.2 E33M58_78
43.1 E42M47_146
45.4 E33M58_45
48.0 CB10448
49.0 E34M54_213
50.0 E33M47_49
51.8 HMR386
52.5 CB10347
53.0 BRAS010a
54.3 E32M50_481
57.4 E45M51_62
66.4 BRAS010b
N5
0.0 CB10545
2.9 E43M52_253
4.0 E32M51_329
5.2 E32M51_325
6.0 E33M50_48
12.4 E44M49_261
17.5 E32M49_149
29.5 E34M59_94
32.8 BRAS063
33.2 HMR342
34.0 Na10E02
36.7 E43M59_132
38.6 E36M47_151
40.5 E36M51_300
41.6 MD21
42.8 CB10574
43.7 E43M52_320
44.0 E34M55_189
45.9 CB10609
47.7 E34M59_153
49.9 E46M62_78
50.7 GMR014
51.5 Na12E01b
53.8 E31M55_105
56.7 E31M62_195
58.7 E33M49_104
60.6 E32M59_162
71.5 E42M55_166
87.8 E42M60_190
N6
0.0 E44M60_472
14.7 E32M48_677
22.0 HMR572
25.8 E33M47_73
36.0 E44M60_432
55.7 E33M60_378
57.5 Na12D08
66.3 E45M49_137
67.7 E33M58_56
70.0 E34M55_290
71.7 E43M62_272
76.5 E33M50_327
78.5 E31M53_161
79.3 HMR558
79.4 HMR201
79.5 HMR389
80.8 E32M62_70
83.0 E43M59_81
85.3 Na12H07b
87.0 E34M62_116
88.2 E34M62_304
93.9 E34M62_305


N7
0.0 E39M49_307
16.4 E44M58_101
22.2 HMR166
24.1 MR153b
25.1 Ra2G08
28.6 Na12B02
35.3 Na12E11
43.7 Ni2D03
N8
0.0 E33M47_216
9.5 E46M59_160
10.8 HMR588a
12.8 E45M57_110
14.3 BRAS039a
16.0 CB10364
16.3 HMR388
16.7 CB10578
16.9 HMR307
18.7 E45M59_514
20.8 HMR353a
21.9 E45M51_344
23.0 E38M55_386
24.0 E31M53_515
30.8 HMR582
31.5 E45M51_302
34.5 E44M48_414
36.9 E32M51_301
41.1 CB10629
42.1 E39M59_106
44.6 E39M61_243
45.4 E43M62_222
52.1 E39M47_181
59.4 Na12B05
68.9 E32M59_123
69.3 E44M49_170
72.5 Ra1F06
77.2 E42M59_122
N9
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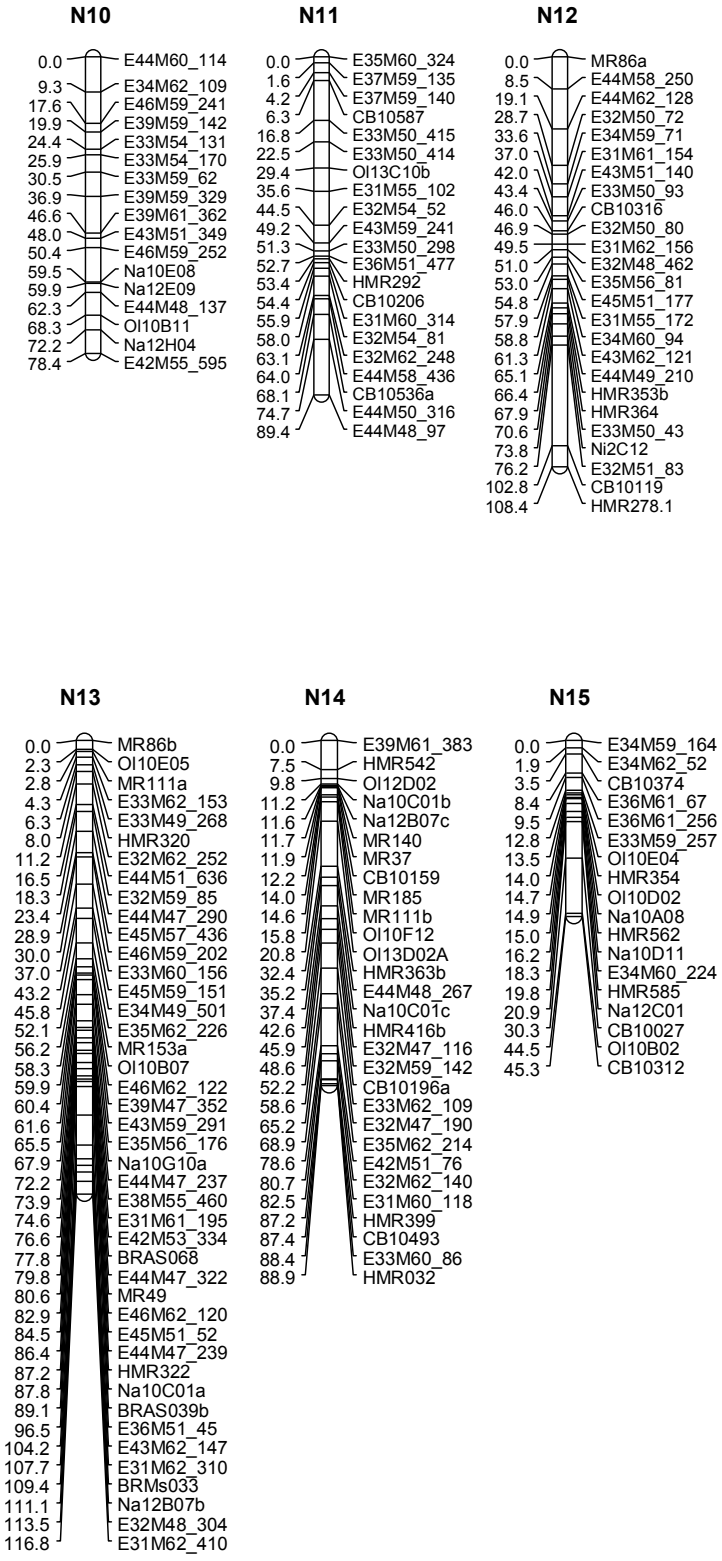
3.0 E44M47_406
3.7 E32M48_148
13.2 E44M51_350
17.9 E35M60_540
22.8 Na10B11
30.4 GMR013a
37.0 E46M59_87
41.9 E38M49_139
43.2 E31M55_333
44.6 HMR360
46.0 BRAS020
47.4 E33M54_331
49.5 Na12E06B
52.4 E33M60_98
53.4 Na14C12
56.7 E36M51_53
57.7 E46M59_79
59.6 E34M49_108
62.0 E36M57_123
62.6 E33M49_170
64.5 E42M59_96
67.1 E31M61_188
68.4 E37M59_237
69.1 HMR602
70.4 E44M57_123
75.0 E45M54_110
75.5 E44M60_80
77.6 MR216
81.4 E42M55_131
92.5 E31M55_241
94.6 E34M51_122
97.6 CB10373
98.8 HMR381
100.2 Na10B07
101.5 E31M55_230
102.6 CB10476
111.6 E33M50_80
115.9 E45M61_233
116.4 E31M53_95
118.2 E32M62_348
120.0 E34M62_196
122.3 E43M62_336
135.3 E33M62_200
136.2 E45M54_187
137.0 MR230
141.9 CB10116b
146.2 E32M48_182
147.0 E31M49_223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36.9 E39M59_329
46.6 E39M61_362
48.0 E43M51_349
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59.5 Na10E08
59.9 Na12E09
62.3 E44M48_137
68.3 OI10B11
72.2 Na12H04
78.4 E42M55_595
N11
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1.6 E37M59_135
4.2 E37M59_140
6.3 CB10587
16.8 E33M50_415
22.5 E33M50_414
29.4 OI13C10b
35.6 E31M55_102
44.5 E32M54_52
49.2 E43M59_241
51.3 E33M50_298
52.7 E36M51_477
53.4 HMR292
54.4 CB10206
55.9 E31M60_314
58.0 E32M54_81
63.1 E32M62_248
64.0 E44M58_436
68.1 CB10536a
74.7 E44M50_316
89.4 E44M48_97
N12
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8.5 E44M58_250
19.1 E44M62_128
28.7 E32M50_72
33.6 E34M59_71
37.0 E31M61_154
42.0 E43M51_140
43.4 E33M50_93
46.0 CB10316
46.9 E32M50_80
49.5 E31M62_156
51.0 E32M48_462
53.0 E35M56_81
54.8 E45M51_177
57.9 E31M55_172
58.8 E34M60_94
61.3 E43M62_121
65.1 E44M49_210
66.4 HMR353b
67.9 HMR364
70.6 E33M50_43
73.8 Ni2C12
76.2 E32M51_83
102.8 CB10119
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0.0 MR86b
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2.8 MR111a
4.3 E33M62_153
6.3 E33M49_268
8.0 HMR320
11.2 E32M62_252
16.5 E44M51_636
18.3 E32M59_85
23.4 E44M47_290
28.9 E45M57_436
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37.0 E33M60_156
43.2 E45M59_151
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59.9 E46M62_122
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65.5 E35M56_176
67.9 Na10G10a
72.2 E44M47_237
73.9 E38M55_460
74.6 E31M61_195
76.6 E42M53_334
77.8 BRAS068
79.8 E44M47_322
80.6 MR49
82.9 E46M62_120
84.5 E45M51_52
86.4 E44M47_239
87.2 HMR322
87.8 Na10C01a
89.1 BRAS039b
96.5 E36M51_45
104.2 E43M62_147
107.7 E31M62_310
109.4 BRMs033
111.1 Na12B07b
113.5 E32M48_304
116.8 E31M62_410
N14
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7.5 HMR542
9.8 OI12D02
11.2 Na10C01b
11.6 Na12B07c
11.7 MR140
11.9 MR37
12.2 CB10159
14.0 MR185
14.6 MR111b
15.8 OI10F12
20.8 OI13D02A
32.4 HMR363b
35.2 E44M48_267
37.4 Na10C01c
42.6 HMR416b
45.9 E32M47_116
48.6 E32M59_142
52.2 CB10196a
58.6 E33M62_109
65.2 E32M47_190
68.9 E35M62_214
78.6 E42M51_76
80.7 E32M62_140
82.5 E31M60_118
87.2 HMR399
87.4 CB10493
88.4 E33M60_86
88.9 HMR032
N15
0.0 E34M59_164
1.9 E34M62_52
3.5 CB10374
8.4 E36M61_67
9.5 E36M61_256
12.8 E33M59_257
13.5 OI10E04
14.0 HMR354
14.7 OI10D02
14.9 Na10A08
15.0 HMR562
16.2 Na10D11
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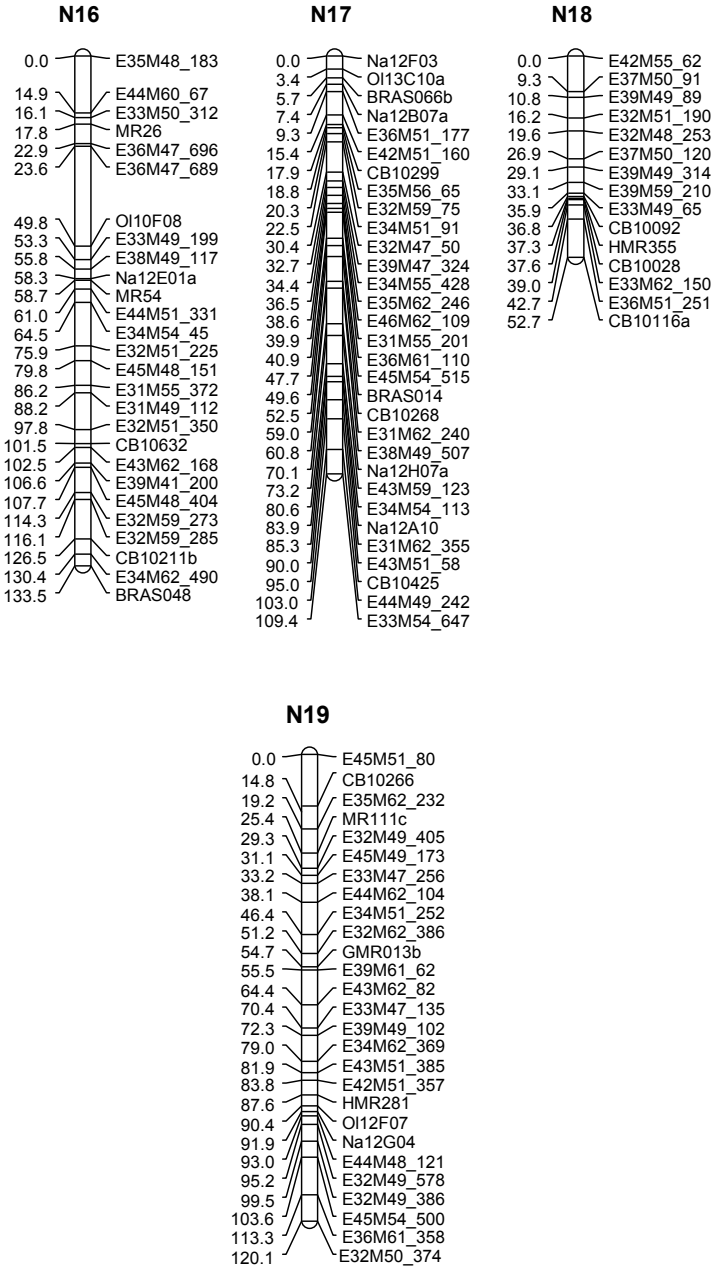
19.8 HMR585
20.9 Na12C01
30.3 CB10027
44.5 OI10B02
45.3 CB10312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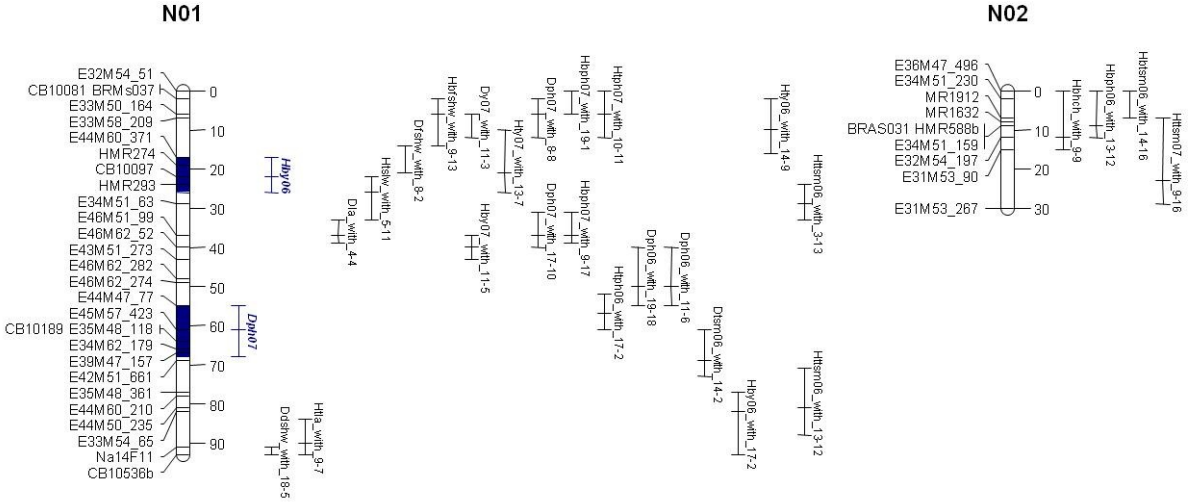
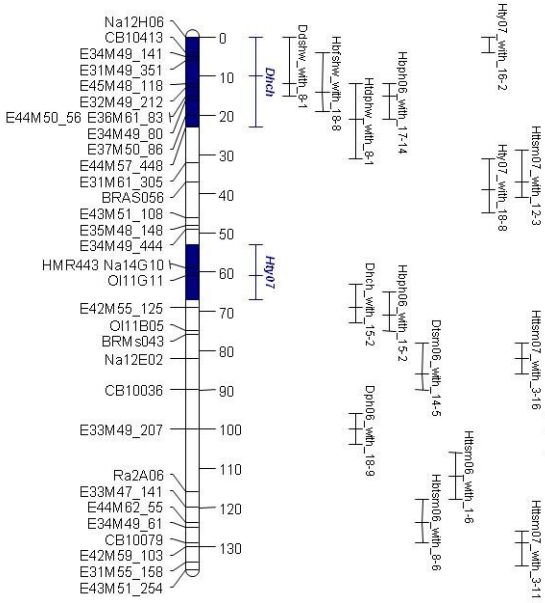
17.8 MR26
22.9 E36M47_696
23.6 E36M47_689
49.8 Ol10F08
53.3 E33M49_199
55.8 E38M49_117
58.3 Na12E01a
58.7 MR54
61.0 E44M51_331
64.5 E34M54_45
75.9 E32M51_225
79.8 E45M48_151
86.2 E31M55_372
88.2 E31M49_112
97.8 E32M51_350
101.5 CB10632
102.5 E43M62_168
106.6 E39M41_200
107.7 E45M48_404
114.3 E32M59_273
116.1 E32M59_285
126.5 CB10211b
130.4 E34M62_490
133.5 BRAS048
N17
0.0 Na12F03
3.4 Ol13C10a
5.7 BRAS066b
7.4 Na12B07a
9.3 E36M51_177
15.4 E42M51_160
17.9 CB10299
18.8 E35M56_65
20.3 E32M59_75
22.5 E34M51_91
30.4 E32M47_50
32.7 E39M47_324
34.4 E34M55_428
36.5 E35M62_246
38.6 E46M62_109
39.9 E31M55_201
40.9 E36M61_110
47.7 E45M54_515
49.6 BRAS014
52.5 CB10268
59.0 E31M62_240
60.8 E38M49_507
70.1 Na12H07a
73.2 E43M59_123
80.6 E34M54_113
83.9 Na12A10
85.3 E31M62_355
90.0 E43M51_58
95.0 CB10425
103.0 E44M49_242
109.4 E33M54_647
N18
0.0 E42M55_62
9.3 E37M50_91
10.8 E39M49_89
16.2 E32M51_190
19.6 E32M48_253
26.9 E37M50_120
29.1 E39M49_314
33.1 E39M59_210
35.9 E33M49_65
36.8 CB10092
37.3 HMR355
37.6 CB10028
39.0 E33M62_150
42.7 E36M51_251
52.7 CB10116a
N19
0.0 E45M51_80
14.8 CB10266
19.2 E35M62_232
25.4 MR111c
29.3 E32M49_405
31.1 E45M49_173
33.2 E33M47_256
38.1 E44M62_104
46.4 E34M51_252
51.2 E32M62_386
54.7 GMR013b
55.5 E39M61_62
64.4 E43M62_82
70.4 E33M47_135
72.3 E39M49_102
79.0 E34M62_369
81.9 E43M51_385
83.8 E42M51_357
87.6 HMR281
90.4 Ol12F07
91.9 Na12G04
93.0 E44M48_121
95.2 E32M49_578
99.5 E32M49_386
103.6 E45M54_500
113.3 E36M61_358
120.1 E32M50_374

Appendix B. Graphical summary of QTL positions

Visual summary of all main-effect and interactive QTL in field and greenhouse trials of 250 DH lines from the cross 'Express 617' x 'V8' and their respective BC test hybrids with 'MSL-Express'. QTL names are derived from the abbreviation of the data set used and the trait observed: D = DH population data, Hb = BC hybrid data, Ht = mid-parent heterosis data, y = yield, ph = plant height, tsm = thousand seed mass, fshw = shoot fresh weight, dshw = shoot dry weight, fphw = photosynthetic organ (leaf) fresh weight, dphw = photosynthetic organ (leaf) dry weight, hch = hypocotyl height, la = leaf area, slw = specific leaf weight.



N03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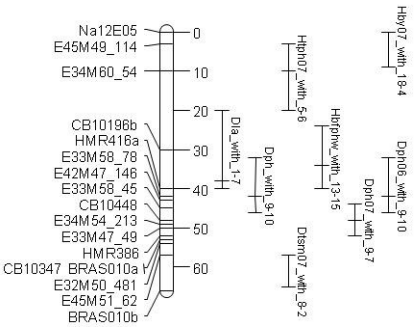
E45M48_118
E32M49_212
E44M50_56 E36M61_83
E34M49_80
E37M50_86
E44M57_448
E31M61_305
BRAS056
E43M51_108
E35M48_148
E34M49_444
HMR443 Na14G10
OI11G11
E42M55_125
OI11B05
BRMs043
Na12E02
CB10036
E33M49_207
Ra2A06
E33M47_141
E44M62_55
E34M49_61
CB10079
E42M59_103
E31M55_158
E43M51_254
0
10
20
30
40
50
60
70
80
90
100
110
120
130
Dhch
Hby07
Ddshw_with_8-1
Hbrshw_with_18-8
Htpphw_with_8-1
Hbph06_with_17-14
Hfy07_with_16-2
Hfy07_with_18-8
Htsm07_with_12-3
Dhch_with_15-2
Hbph06_with_15-2
Dtsm06_with_14-5
Dph06_with_18-9
Htsm06_with_1-6
Hdtsm06_with_8-6
Hbph07_with_7-5
Hbph07_with_17-11
Dph07_with_11-2
Htdsm07_with_16-9
Hby07_with_19-11
Htsm07_with_3-16
Dy06_with_9-23
Dtsm07_with_9-7
Htsm07_with_3-11

N04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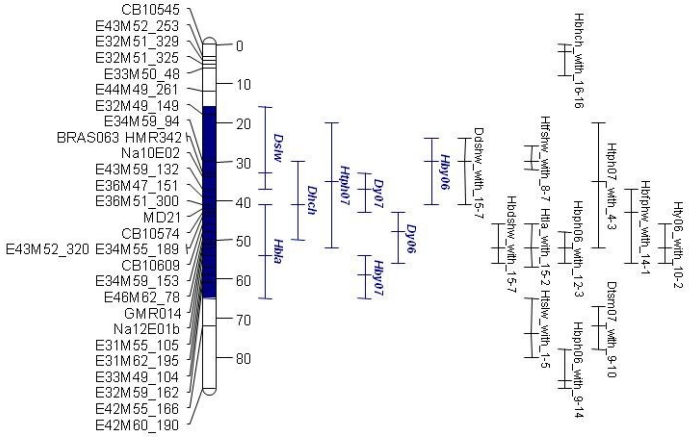
CB10196b
HMR416a
E33M58_78
E42M47_146
E33M58_45
CB10448
E34M54_213
E33M47_49
HMR386
CB10347 BRAS010a
E32M50_481
E45M51_62
BRAS010b
0
10
20
30
40
50
60
Hby07_with_18-4
Hbph07_with_5-6
Htrphw_with_13-15
Dph07_with_9-7
Dph06_with_9-10
Dtsm07_with_8-2
Dla_with_1-7
Dph_with_9-10

N05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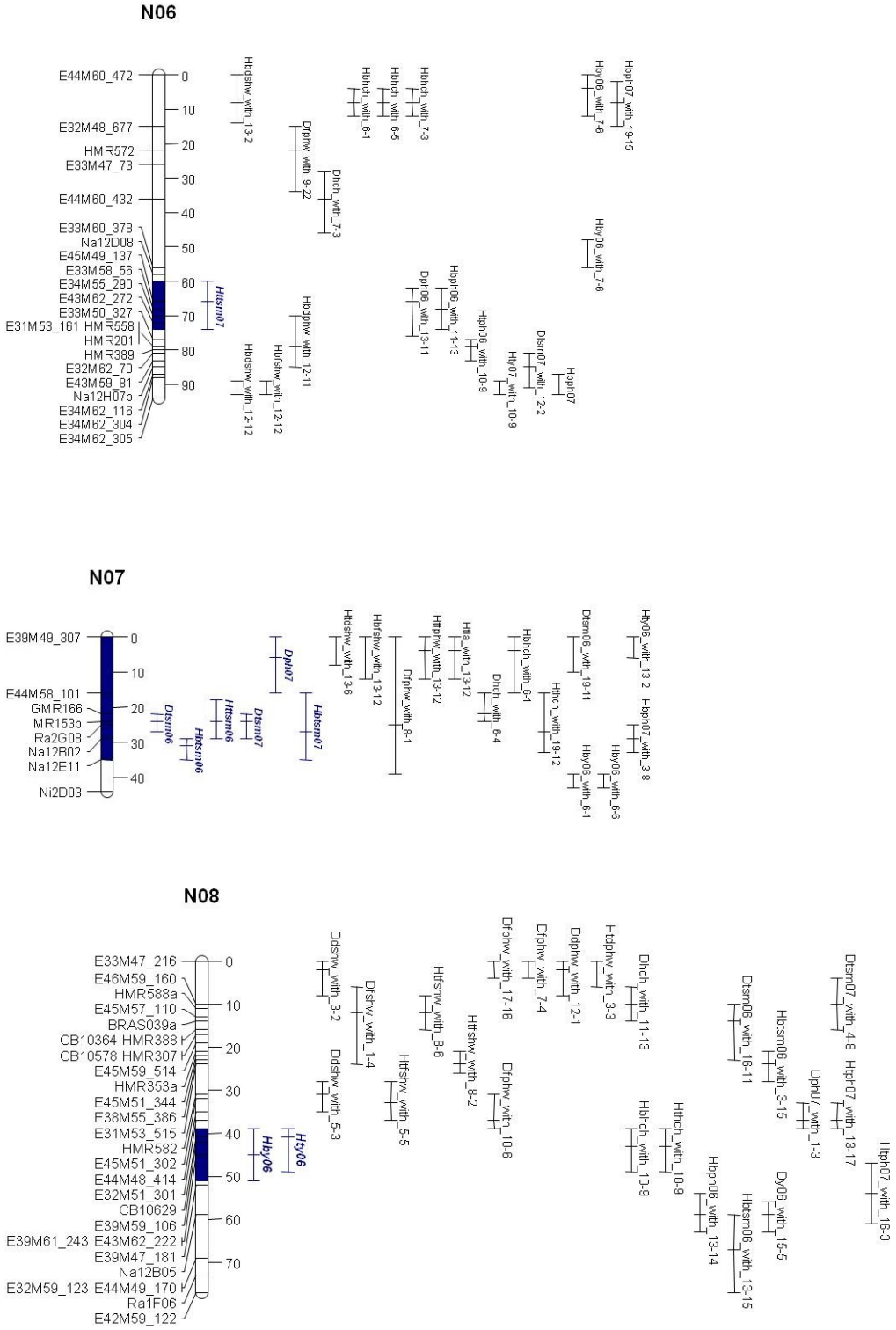
E32M51_325
E33M50_48
E44M49_261
E32M49_149
E34M59_94
BRAS063 HMR342
Na10E02
E43M59_132
E36M47_151
E36M51_300
MD21
CB10574
E43M52_320 E34M55_189
CB10609
E34M59_153
E46M62_78
GMR014
Na12E01b
E31M55_105
E31M62_195
E33M49_104
E32M59_162
E42M55_166
E42M60_190
0
10
20
30
40
50
60
70
80
Dstw
Dhch
Hbla
Hbph07
Dy07
Hby07
Hby06
Dy06
Hbhch_with_16-16
Htrshw_with_8-7
Htla_with_15-2
Htslw_with_1-5
Hbdshw_with_15-7
Ddshw_with_15-7
Hbph06_with_12-3
Hbph07_with_4-3
Htrphw_with_14-1
Hby06_with_10-2
Dtsm07_with_9-10
Hbph06_with_9-14

Appendix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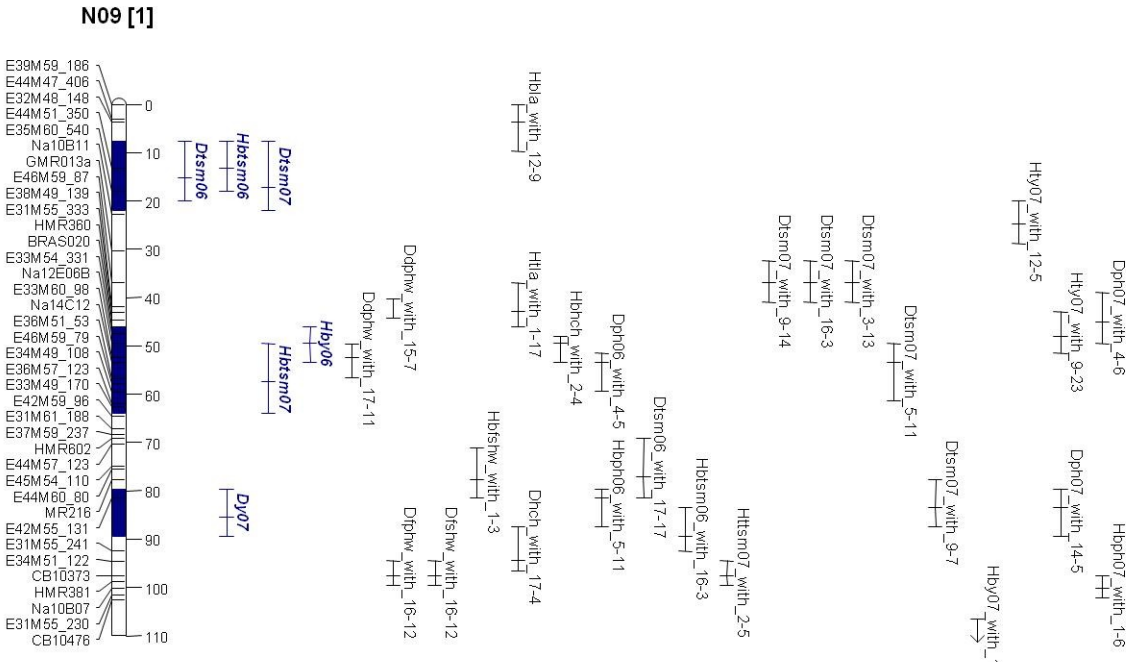
E33M58_56
E34M55_290
E43M62_272
E33M50_327
E31M53_161 HMR558
HMR201
HMR389
E32M62_70
E43M59_81
Na12H07b
E34M62_116
E34M62_304
E34M62_305
Httsm07
Hbdshw_with_13-2
Dfphw_with_9-22
Dhch_with_7-3
Hbhch_with_8-1
Hbhch_with_6-5
Hbhch_with_7-3
Hbphn07_with_19-15
Hby06_with_7-6
Hby06_with_7-6
Hbtdphw_with_12-11
Htdshw_with_12-12
Htfshw_with_12-12
Dph06_with_13-11
Hbphn06_with_11-13
Hbphn06_with_10-9
Hby07_with_10-9
Dtsm07_with_12-2
Hbphn07
N07
E39M49_307
E44M58_101
GMR166
MR153b
Ra2G08
Na12B02
Na12E11
Ni2D03
Dtsm06
Htbtsm06
Htsm06
Dtsm07
Dph07
Httsm07
Htdshw_with_13-6
Htfshw_with_13-12
Htfphw_with_13-12
Hfa_with_13-12
Dfphw_with_8-1
Dhch_with_6-4
Hthch_with_6-1
Hthch_with_19-12
Dtsm06_with_19-11
Hby06_with_6-1
Hby06_with_6-6
Hby06_with_13-2
Hbphn07_with_3-8
N08
E33M47_216
E46M59_160
HMR588a
E45M57_110
BRAS039a
CB10364 HMR388
CB10578 HMR307
E45M59_514
HMR353a
E45M51_344
E38M55_386
E31M53_515
HMR582
E45M51_302
E44M48_414
E32M51_301
CB10629
E39M59_106
E39M61_243 E43M62_222
E39M47_181
Na12B05
E32M59_123 E44M49_170
Ra1F06
E42M59_122
Hty06
Hby06
Ddshw_with_3-2
Dfshw_with_1-4
Ddshw_with_5-3
Htfshw_with_5-5
Htfshw_with_8-6
Htfshw_with_8-2
Dfphw_with_17-16
Dfphw_with_10-6
Dfphw_with_7-4
Ddphw_with_12-1
Htdphw_with_3-3
Dhch_with_11-13
Hbhch_with_10-9
Hthch_with_10-9
Hbphn06_with_13-14
Dtsm06_with_16-11
Htbtsm06_with_13-15
Htbtsm06_with_3-15
Dy06_with_15-5
Dph07_with_1-3
Dtsm07_with_4-8
Htphn07_with_13-17
Htphn07_with_16-3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E46M59_87
E38M49_139
E31M55_333
HMR360
BRAS020
E33M54_331
Na12E06B
E33M60_98
Na14C12
E36M51_53
E46M59_79
E34M49_108
E36M57_123
E33M49_170
E42M59_96
E31M61_188
E37M59_237
HMR602
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E45M54_110
E44M60_80
MR216
E42M55_131
E31M55_241
E34M51_122
CB10373
HMR381
Na10B07
E31M55_230
CB10476
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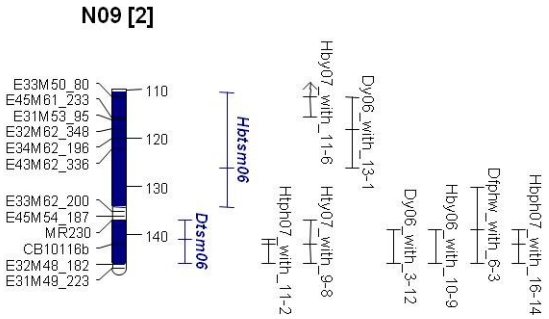
10
20
30
40
50
60
70
80
90
100
110
Dtsm07
Hbtsm06
Dtsm06
Hby06
Hbtsm07
Dy07
Hbla_with_12-9
Htla_with_1-17
Hbhrch_with_2-4
Dqphw_with_15-7
Dqphw_with_17-11
Hbfshw_with_1-3
Dfshw_with_16-12
Dfphw_with_16-12
Dhch_with_17-4
Dph06_with_4-5
Hbph06_with_5-11
Dtsm06_with_17-17
Hbtsm06_with_16-3
Httsm07_with_2-5
Dtsm07_with_9-14
Dtsm07_with_16-3
Dtsm07_with_3-13
Dtsm07_with_5-11
Dtsm07_with_9-7
Hby07_with_11-6
Hty07_with_12-5
Dph07_with_4-6
Hty07_with_9-23
Dph07_with_14-5
Hbph07_with_1-6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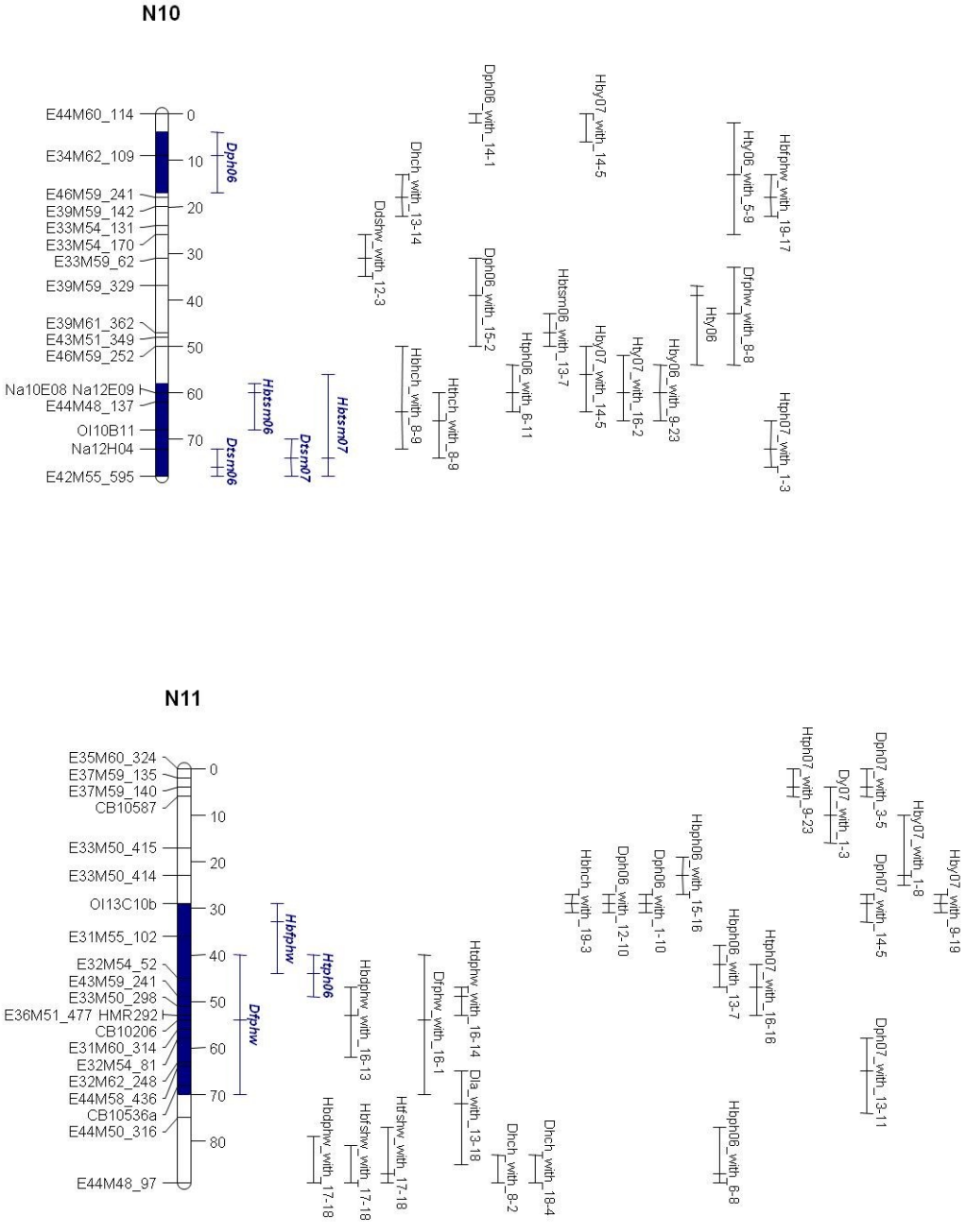
E32M62_348
E34M62_196
E43M62_336
E33M62_200
E45M54_187
MR230
CB10116b
E32M48_182
E31M49_223
110
120
130
140
Hbtsm06
Dtsm06
Hby07_with_11-6
Dy06_with_13-1
Htph07_with_11-2
Hty07_with_9-8
Dy06_with_3-12
Dfphw_with_6-3
Hby06_with_10-9
Hbph07_with_16-14

Appendix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E39M61_362
E43M51_349
E46M59_252
Na10E08 Na12E09
E44M48_137
OI10B11
Na12H04
E42M55_595
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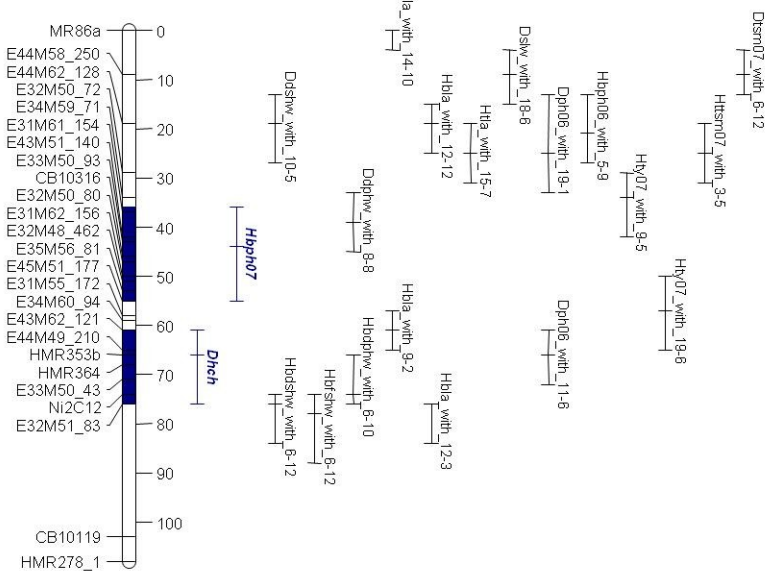
30
40
50
60
70
Dph06
Htstsm06
Dtsm07
Dtsm06
Htstsm07
Dph06_with_14-1
Dhch_with_13-14
Ddshw_with_12-3
Dph06_with_15-2
Hbhch_with_8-9
Htchh_with_8-9
Htph06_with_6-11
Hby07_with_14-5
Hbtsm06_with_13-7
Hby07_with_14-5
Hby07_with_16-2
Hby06_with_9-23
Hby06
Dfphw_with_8-8
Hby06_with_5-9
Hbfphw_with_19-17
Htph07_with_1-3
N11
E35M60_324
E37M59_135
E37M59_140
CB10587
E33M50_415
E33M50_414
OI13C10b
E31M55_102
E32M54_52
E43M59_241
E33M50_298
E36M51_477 HMR292
CB10206
E31M60_314
E32M54_81
E32M62_248
E44M58_436
CB10536a
E44M50_316
E44M48_97
0
10
20
30
40
50
60
70
80
Hbfphw
Htph06
Dfphw
Hbdphw_with_16-13
Hbdphw_with_17-18
Hbfshw_with_17-18
Htfshw_with_17-18
Htdphw_with_16-14
Dfphw_with_16-1
Dla_with_13-18
Dhch_with_8-2
Dhch_with_18-4
Hbhch_with_19-3
Dph06_with_12-10
Dph06_with_1-10
Htph06_with_15-16
Htph06_with_13-7
Htph07_with_16-16
Hbph06_with_6-8
Htph07_with_9-23
Dy07_with_1-3
Dph07_with_3-5
Hby07_with_1-8
Dph07_with_14-5
Dph07_with_13-11
Hby07_with_9-19

N12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E32M50_72
E34M59_71
E31M61_154
E43M51_140
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CB10316
E32M50_80
E31M62_156
E32M48_462
E35M56_81
E45M51_177
E31M55_172
E34M60_94
E43M62_121
E44M49_210
HMR353b
HMR364
E33M50_43
Ni2C12
E32M51_83
CB10119
HMR278_1
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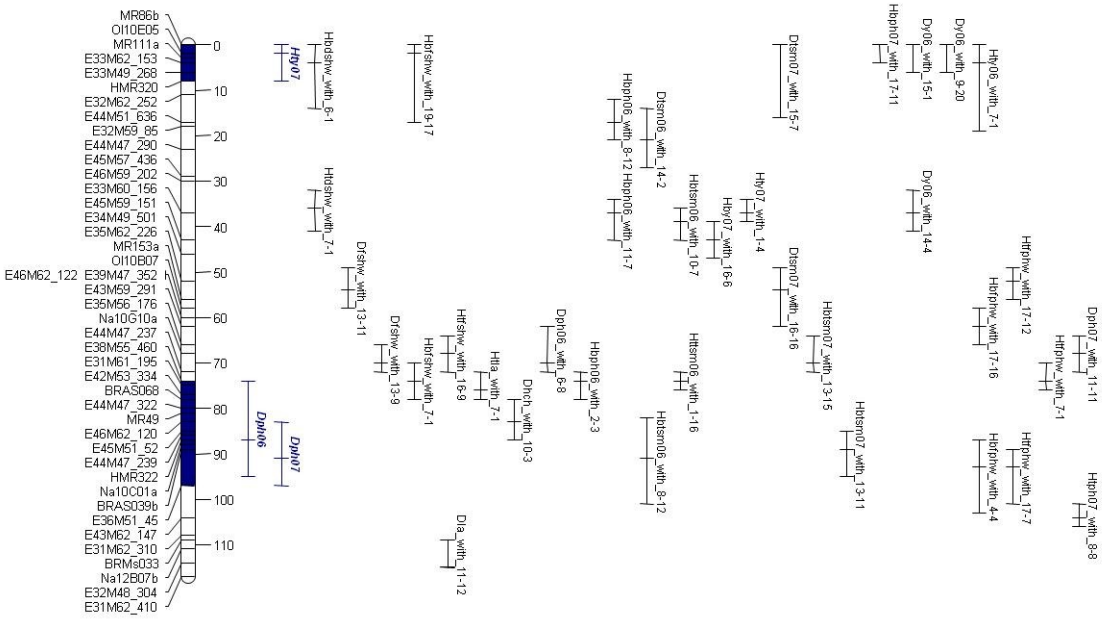
20
30
40
50
60
70
80
90
100
Hbph07
Dhch
Ddshw_with_10-5
Dla_with_14-10
Ddqhw_with_8-8
Hbla_with_12-12
Hbdshw_with_6-12
Hbdqhw_with_6-10
Hbfshw_with_8-12
Hbla_with_9-2
Hbla_with_12-3
Htla_with_15-7
Dslw_with_18-6
Dph06_with_19-1
Dph06_with_11-6
Hbph06_with_5-9
Hby07_with_9-5
Hby07_with_19-6
Dtsm07_with_6-12
Httsm07_with_3-5

N13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MR111a
E33M62_153
E33M49_268
HMR320
E32M62_252
E44M51_636
E32M59_85
E44M47_290
E45M57_436
E46M59_202
E33M60_156
E45M59_151
E34M49_501
E35M62_226
MR153a
OI10B07
E46M62_122
E39M47_352
E43M59_291
E35M56_176
Na10G10a
E44M47_237
E38M55_460
E31M61_195
E42M53_334
BRAS068
E44M47_322
MR49
E46M62_120
E45M51_52
E44M47_239
HMR322
Na10C01a
BRAS039b
E36M51_45
E43M62_147
E31M62_310
BRMs033
Na12B07b
E32M48_304
E31M62_410
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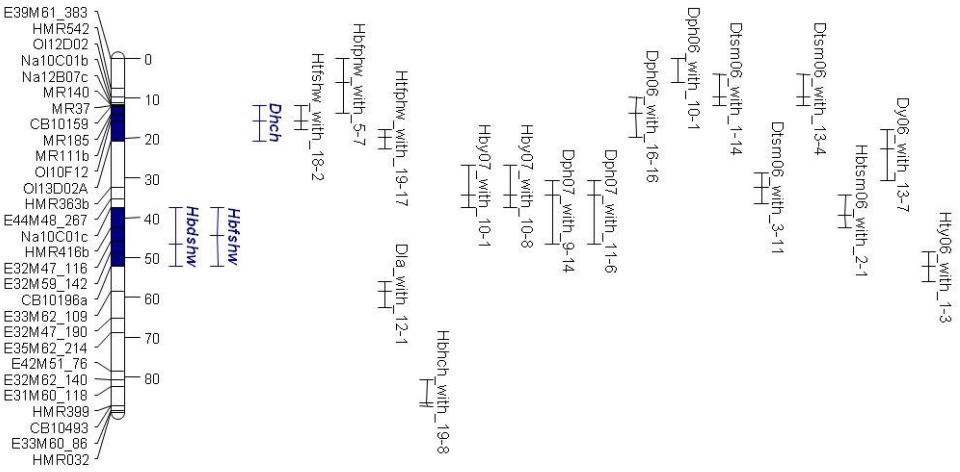
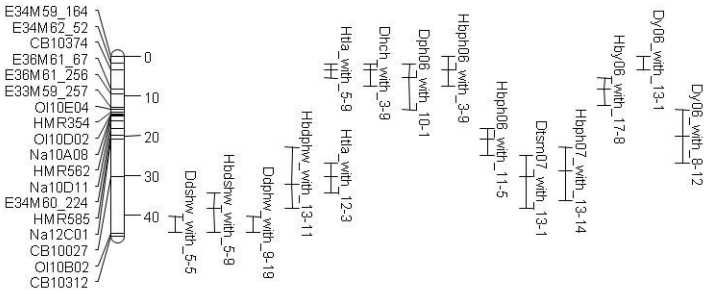
60
70
80
90
100
110
Hby07
Dph06
Dph07
Hbdshw_with_6-1
Htdshw_with_7-1
Dfshw_with_13-11
Dfshw_with_13-9
Hbfshw_with_19-17
Hbfshw_with_7-1
Htfshw_with_16-9
Htla_with_7-1
Dla_with_11-12
Dhch_with_10-3
Dph06_with_8-8
Hbph06_with_2-3
Hbph06_with_8-12
Hbph06_with_11-7
Dtsm06_with_14-2
Hbtsm06_with_10-7
Hby07_with_1-4
Hby07_with_16-6
Hbtsm06_with_1-16
Hbtsm06_with_8-12
Dtsm07_with_15-7
Dtsm07_with_16-16
Hbtsm07_with_13-15
Hbtsm07_with_13-11
Hbph07_with_17-11
Dy06_with_15-1
Dy06_with_9-20
Hby06_with_7-1
Dy06_with_14-4
Hbfphw_with_17-16
Htfphw_with_17-12
Htfphw_with_7-1
Dph07_with_11-11
Hbfphw_with_4-4
Htfphw_with_17-7
Hbph07_with_8-8

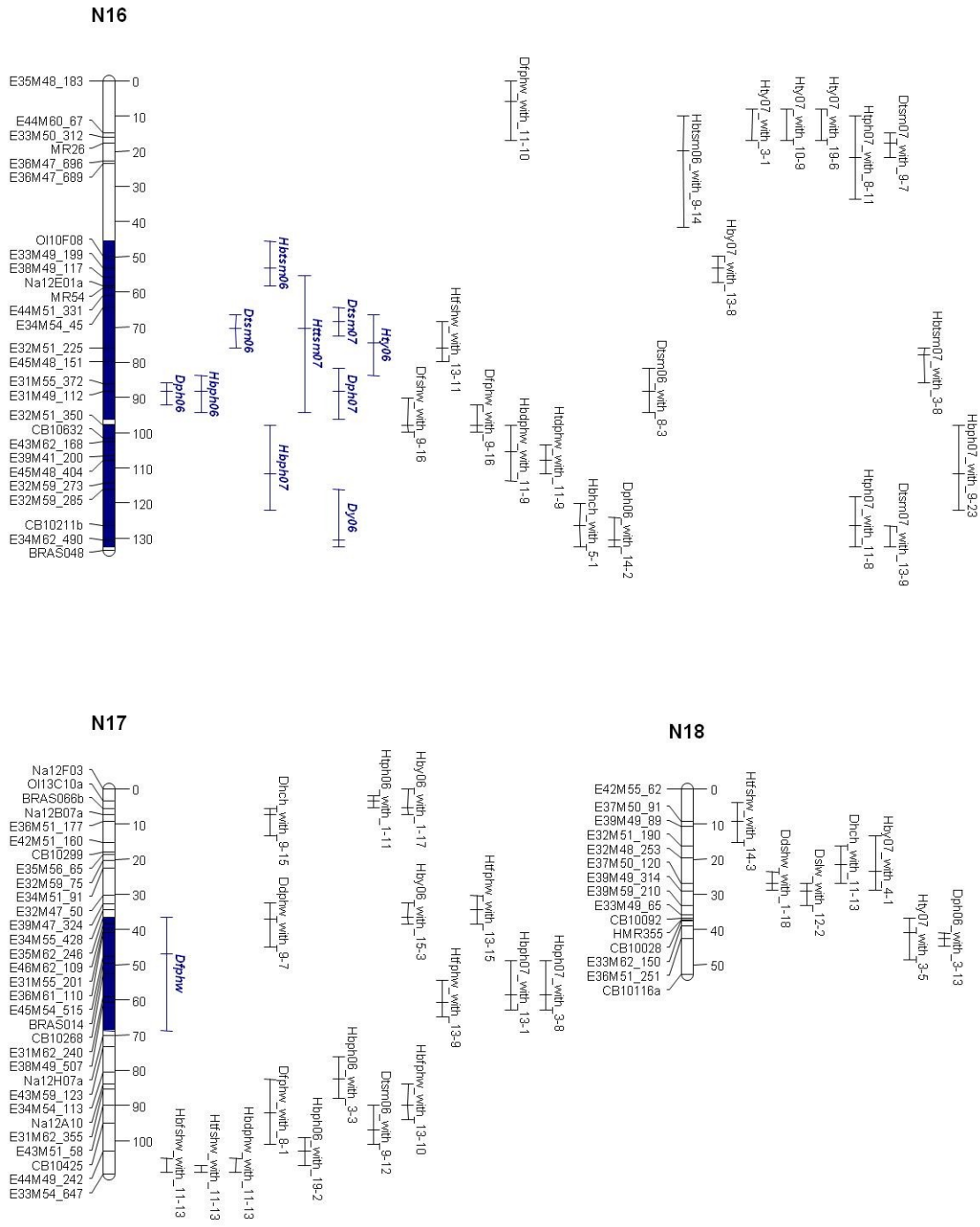
Appendix

N14



N15

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E44M60_67
E33M50_312
MR26
E36M47_696
E36M47_689
OI10F08
E33M49_199
E38M49_117
Na12E01a
MR54
E44M51_331
E34M54_45
E32M51_225
E45M48_151
E31M55_372
E31M49_112
E32M51_350
CB10632
E43M62_168
E39M41_200
E45M48_404
E32M59_273
E32M59_285
CB10211b
E34M62_490
BRAS048
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80
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120
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Htstsm06
Htstsm07
Dtsm06
Dtsm07
Hty06
Dph07
Hbph06
Dph06
Hbph07
Dy06
Dfphw_with_11-10
Htfshw_with_13-11
Dfshw_with_9-16
Dfphw_with_9-16
Htdphw_with_11-9
Hbdphw_with_11-9
Htdphw_with_11-9
Hbhch_with_5-1
Dtsm06_with_8-3
Dph06_with_14-2
Htstsm06_with_9-14
Hby07_with_13-8
Hby07_with_3-1
Hby07_with_10-9
Hby07_with_19-6
Htstsm07_with_3-8
Htph07_with_8-11
Dtsm07_with_9-7
Htph07_with_11-8
Dtsm07_with_13-9
Htph07_with_9-23
N17
Na12F03
OI13C10a
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Na12B07a
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E42M51_160
CB10299
E35M56_65
E32M59_75
E34M51_91
E32M47_50
E39M47_324
E34M55_428
E35M62_246
E46M62_109
E31M55_201
E36M61_110
E45M54_515
BRAS014
CB10268
E31M62_240
E38M49_507
Na12H07a
E43M59_123
E34M54_113
Na12A10
E31M62_355
E43M51_58
CB10425
E44M49_242
E33M54_647
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60
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80
90
100
Dfphw
Htfshw_with_11-13
Htfshw_with_11-13
Hbdphw_with_11-13
Dfphw_with_8-1
Hbph06_with_19-2
Dtsm06_with_9-12
Htph06_with_3-3
Hbrphw_with_13-10
Htfphw_with_13-9
Htfphw_with_13-15
Hbph07_with_13-1
Hbph07_with_3-8
Htph06_with_1-11
Hby06_with_1-17
Hby06_with_15-3
Dhch_with_9-15
Ddphw_with_9-7
N18
E42M55_62
E37M50_91
E39M49_89
E32M51_190
E32M48_253
E37M50_120
E39M49_314
E39M59_210
E33M49_65
CB10092
HMR355
CB10028
E33M62_150
E36M51_251
CB10116a
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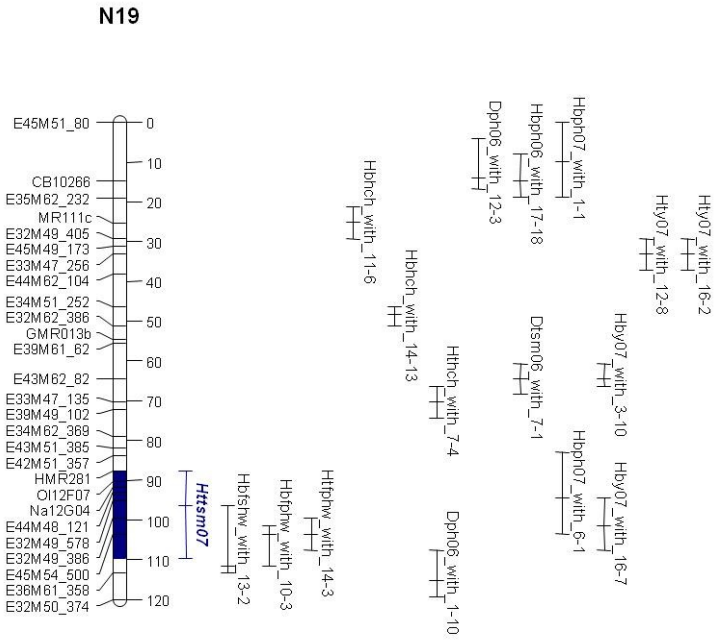
Htfshw_with_14-3
Ddshw_with_1-18
Dhch_with_11-13
Dslw_with_12-2
Hby07_with_4-1
Hty07_with_3-5
Dph06_with_3-13

Appendix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E32M49_405
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E33M47_256
E44M62_104
E34M51_252
E32M62_386
GMR013b
E39M61_62
E43M62_82
E33M47_135
E39M49_102
E34M62_369
E43M51_385
E42M51_357
HMR281
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Na12G04
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E32M49_578
E32M49_386
E45M54_500
E36M61_358
E32M50_374
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70
80
90
100
110
120
Httsm07
Hbfshw_with_13-2
Hbfphw_with_10-3
Httphw_with_14-3
Hbhch_with_11-6
Hbhch_with_14-13
Hthch_with_7-4
Dph06_with_1-10
Dph06_with_12-3
Hbphh07_with_6-1
Hby07_with_16-7
Hbphh06_with_17-18
Dtsm06_with_7-1
Hbphh07_with_1-1
Hby07_with_3-10
Hty07_with_12-8
Hty07_with_16-2

Appendix C. List of epistatic QTL

Epistatic (interactive) QTL that were detected in this study. QTL names are derived from the abbreviation of the data set used and the trait observed: D = DH population data, Hb = BC hybrid data, Ht = mid-parent heterosis data, fshw = shoot fresh weight, dshw = shoot dry weight, fphw = photosynthetic organ (leaf) fresh weight, dphw = photosynthetic organ (leaf) dry weight, hch = hypocotyl height, la = leaf area, slw = specific leaf weight. y = yield, ph = plant height, tsm = thousand seed mass. Values of genetic effects are presented after adjustments for BC and MP: Effects of QTL obtained from all the data set were not adjusted.

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Ddshw N01-N18	Na14F11	CB10536b	92.5	90.5-92.5	E37M50120	E39M49314	26.9	23.6-28.9	0.03	5
Ddshw N03-N08	CB10413	E36M6183	11.5	0.0-14.5	E33M47216	E46M59160	2.0	0.0-8.0	0.02	4
Ddshw N05-N08	E44M49261	E32M49149	12.4	6.0-14.4	HMR582	E32M51301	30.8	28.0-34.8	-0.02	3
Ddshw N05-N15	E34M5994	BRAS063	29.5	23.5-40.8	CB10027	CB10312	44.3	40.3-44.3	-0.02	5
Ddshw N10-N12	E33M5962	E39M59329	30.5	26.4-34.5	E44M62128	E32M5072	19.1	12.5-27.1	-0.02	3
Ddphw N08-N12	E32M51301	CB10629	38.9	32.8-45.1	MR86a	E44M58250	2.0	0.0-8.0	-0.01	6
Ddphw N09-N17	E46M5987	BRAS020	37.0	32.4-45.0	CB10268	E38M49507	52.5	49.6-56.5	0.01	7
Ddphw N09-N15	E33M5080	E31M5395	111.6	102.6-118.4	CB10027	CB10312	44.3	40.3-44.3	0.01	4
Dfshw N01-N08	E44M60371	CB10097	20.9	14.0-20.9	E46M59160	BRAS039a	11.5	6.0-24.0	0.313	3.5
Dfshw N09-N16	CB10373	Na10B07	97.6	94.5-99.6	E32M51350	CB10632	97.8	90.2-99.8	0.362	4.8
Dfshw N13-N13	E35M62226	Ol10B07	54.1	49.2-58.3	Na10G10a	E38M55460	69.9	66.3-71.9	-0.457	7.6
Dfphw N06-N09	HMR572	E33M4773	22.0	14.7-33.8	MR230	CB10116b	139.0	130.3-145.9	-0.127	3.0
Dfphw N07-N08	Ra2G08	Na12B02	25.1	0.0-39.3	E33M47216	E46M59160	0.0	0.0-4.0	-0.112	2.3

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Dfphw N08-N17	E33M47216	E46M59160	0.0	0.0-4.0	E43M5158	CB10425	92.0	82.6-101.0	-0.187	6.6
Dfphw N08-N10	E32M51301	CB10629	36.9	30.8-38.9	E39M59329	E39M61362	42.9	32.5-54.4	0.138	3.6
Dfphw N09-N16	CB10373	Na10B07	97.6	94.5-99.6	E32M51350	CB10632	97.8	92.2-99.8	0.179	6.0
Dfphw N11-N16	CB10206	E32M62248	54.4	39.6-70.1	E35M48183	E44M6067	6.0	0.0-16.9	0.134	3.4
Dhch N03-N15	E42M55125	BRMS043	68.7	63.2-72.7	CB10374	E36M6167	3.5	2.0-7.5	2.438	6.3
Dhch N06-N07	E33M4773	E44M60432	35.8	27.8-46.0	GMR166	Ra2G08	22.2	16.4-24.2	1.883	3.8
Dhch N08-N11	E46M59160	BRAS039a	9.5	6.0-13.5	E44M50316	E44M4897	88.7	82.7-88.7	-1.833	3.6
Dhch N09-N17	E31M55249	CB10373	94.5	87.4-96.5	Na12B07a	CB10299	7.4	5.7-13.4	2.171	5
Dhch N10-N13	E46M59241	E33M54131	17.6	13.3-21.6	E46M62120	BRAS039b	82.9	77.8-86.9	-1.901	3.8
Dhch N11-N18	E44M50316	E44M4897	88.7	82.7-88.7	E32M48253	E37M50120	21.6	16.2-26.9	1.858	3.7
Dla N01-N04	E46M5199	E46M6252	36.8	32.5-38.8	CB10196b	HMR416a	37.6	20.2-39.9	-14.25	4
Dla N11-N13	CB10536a	E44M50316	72.1	65.1-84.7	BRMS033	E31M62410	115.4	109.4-115.4	-16.20	5
Dla N12-N14	MR86a	E44M58250	0.0	0.0-4.0	E33M62109	E32M47190	58.6	56.2-62.6	-16.10	5

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Dslw N12-N18	E44M58250	E44M62128	8.5	4.0-14.5	E39M49314	CB10092	29.1	26.9-33.1	0.038	4.4
Dy07 N01-N11	E33M50164	E44M60371	6.0	6.0-12.0	CB10587	E33M50415	10.3	4.2-16.3	-0.71	4
Dph07 N01-N08	E33M50164	E44M60371	6.0	1.7-12.0	E32M51301	CB10629	36.9	32.8-38.9	-1.47	3
Dph07 N01-N17	E46M5199	E46M6252	36.8	30.5-39.6	BRAS014	CB10268	51.6	44.9-60.5	-1.19	2
Dph07 N03-N11	BRAS056	E43M51108	44.6	36.6-48.4	E37M59140	CB10587	4.2	0.0-6.2	1.58	4
Dph07 N04-N09	CB10448	CB10347	48.0	43.9-52.0	E46M5987	BRAS020	45.0	39.0-49.5	-1.83	5
Dph07 N09-N14	E42M55131	E31M55249	83.4	79.6-89.4	HMR363b	Na10C01c	34.4	30.8-46.6	1.61	4
Dph07 N11-N14	Ol13C10b	E31M55102	29.4	26.5-33.4	HMR363b	Na10C01c	34.4	30.8-46.6	-1.10	2
Dph07 N11-N13	E32M62248	CB10536a	65.1	58.4-74.1	Na10G10a	E38M55460	67.9	64.3-71.9	-1.32	3
Dtsm07 N03-N09	E33M49207	Ra2A06	99.8	95.8-105.8	E46M5987	BRAS020	37.0	32.4-41.0	-0.07	3
Dtsm07 N04-N08	E45M5162	BRAS010b	65.4	57.4-65.4	E46M59160	BRAS039a	9.5	4.0-16.0	-0.06	2
Dtsm07 N05-N09	E42M55166	E42M60190	71.5	66.7-77.5	Na14C12	E36M57123	53.4	49.5-61.4	0.06	2
Dtsm07 N06-N12	Na12H07b	E34M62305	85.3	81.3-91.3	E44M58250	E44M62128	8.5	4.0-12.5	-0.06	2

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Dtsm07 N09-N09	E46M5987	BRAS020	37.0	32.4-41.0	E42M55131	E31M55249	83.4	77.6-87.4	-0.05	2
Dtsm07 N09-N16	E46M5987	BRAS020	37.0	32.4-41.0	MR26	E36M47689	17.8	14.9-21.8	0.06	2
Dtsm07 N13-N15	MR86b	Ol10E05	0.0	0.0-16.0	CB10027	CB10312	30.3	24.9-38.3	-0.05	1
Dtsm07 N13-N16	E35M62226	Ol10B07	54.1	49.2-62.3	CB10211b	BRAS048	132.5	126.5-132.5	0.06	2
Dy06 N03-N09	CB10036	E33M49207	93.8	84.0-99.8	CB10116b	E31M49223	145.9	139.0-145.9	1.18	10
Dy06 N08-N15	Na12B05	E32M59123	59.4	56.1-63.4	Na10D11	Na12C01	20.2	13.5-26.9	-0.90	6
Dy06 N09-N13	E31M5395	E43M62336	118.4	111.6-126.3	MR86b	Ol10E05	0.0	0.0-6.3	-0.60	2
Dy06 N13-N15	MR86b	Ol10E05	0.0	0.0-6.3	E34M59164	CB10374	0.0	0.0-3.5	0.56	2
Dy06 N13-N14	E33M60156	E45M59151	37.0	32.0-41.0	Ol13D02a	HMR363b	22.8	17.8-30.8	-0.83	5
Dtsm06 N01-N14	E42M51661	E35M48361	68.6	60.9-72.6	Ol12D02	Ol10F12	9.8	4.0-11.8	0.062	2.5
Dtsm06 N03-N14	Na12E02	CB10036	86.0	77.7-89.8	HMR363b	Na10C01c	32.4	28.8-36.4	0.064	2.7
Dtsm06 N07-N19	E39M49307	E44M58101	0.0	0.0-10.0	E43M6282	E33M47135	64.4	60.7-68.4	-0.076	3.8
Dtsm06 N08-N16	BRAS039a	CB10364	14.3	9.5-22.8	E31M49112	E32M51350	88.2	81.8-94.2	-0.052	1.8

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Dtsm06 N09-N17	HMR602	MR216	77.1	69.1-81.4	CB10425	E44M49242	97.0	90.0-101.0	0.062	2.6
Dtsm06 N13-N14	E44M51636	E44M47290	20.5	14.0-27.4	Ol12D02	Ol10F12	9.8	4.0-11.8	-0.053	1.9
Dph06 N01-N19	E46M62282	E44M4777	49.6	39.6-54.8	E36M61358	E32M50374	115.3	107.6-119.3	-1.067	2.7
Dph06 N01-N11	E46M62282	E44M4777	49.6	39.6-54.8	Ol13C10b	E31M55102	29.4	26.5-31.4	1.001	2.4
Dph06 N03-N18	E33M49207	Ra2A06	99.8	95.8-103.8	E36M51251	CB10116a	42.7	41.0-44.7	-1.158	3.2
Dph06 N04-N09	HMR416a	CB10448	41.9	31.6-45.9	Na14C12	E36M57123	53.4	51.5-59.4	-1.028	2.5
Dph06 N06-N13	E45M49137	E34M55290	66.3	61.5-76.0	Na10G10a	E38M55460	69.9	62.3-71.9	-1.311	4.1
Dph06 N10-N14	E44M60114	E34M62109	0.0	0.0-2.0	E39M61383	Ol12D02	0.0	0.0-6.00	1.160	3.2
Dph06 N10-N15	E39M59329	E39M61362	38.9	30.5-50.4	CB10374	E36M6167	5.5	2.0-13.5	1.140	3.1
Dph06 N11-N12	Ol13C10b	E31M55102	29.4	26.5-31.4	HMR353b	Ni2C12	66.4	61.3-72.4	-0.786	1.5
Dph06 N12-N19	E44M62128	E32M5072	25.1	12.5-32.7	E45M5180	CB10266	14.0	4.0-16.8	1.066	2.7
Dph06 N14-N16	Ol12D02	Ol10F12	13.8	9.8-19.8	CB10211b	BRAS048	130.5	124.1-132.5	1.377	4.6

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hbdshw N05-N15	Na12E01b	E31M62195	51.5	45.9-55.5	CB10027	CB10312	38.3	34.3-44.3	-0.02	6
Hbdshw N06-N13	E44M60472	E32M48677	8.0	0.0-14.0	Ol10E05	HMR320	4.3	0.0-14.0	-0.02	7
Hbdshw N06-N12	Na12H07b	E34M62305	93.3	89.3-93.3	E32M5183	CB10119	76.2	73.8-84.2	-0.03	11
Hbdphw N06-N12	E33M50327	HMR558	78.5	70.0-85.3	Ni2C12	E32M5183	73.8	66.4-75.8	-0.01	3
Hbdphw N11-N16	E43M59241	CB10206	53.2	46.5-62.4	CB10632	E45M48404	105.5	97.8-113.7	-0.01	5
Hbdphw N11-N17	E44M50316	E44M4897	88.7	78.7-88.7	E44M49242	E33M54647	109.0	105.0-109.0	-0.02	11
Hbdphw N13-N15	Na10G10a	E38M55460	67.9	64.3-69.9	CB10027	CB10312	32.3	22.9-38.3	-0.01	5
Hbfshw N01-N09	E33M50164	E44M60371	6.0	1.7-14.0	MR216	E42M55131	77.6	71.1-81.4	-0.280	5.3
Hbfshw N03-N18	CB10413	E36M6183	13.5	3.5-18.5	E33M62150	E36M51251	39.0	23.6-42.7	0.242	4
Hbfshw N06-N12	Na12H07b	E34M62305	93.3	89.3-93.3	E32M5183	CB10119	78.2	73.8-88.2	-0.263	4.7
Hbfshw N07-N13	E39M49307	E44M58101	0.0	0.0-12.0	E38M55460	BRAS068	73.9	69.9-77.8	0.241	3.9
Hbfshw N11-N17	E44M50316	E44M4897	88.7	80.7-88.7	E44M49242	E33M54647	109.0	105.0-109.0	-0.291	5.7
Hbfshw N13-N19	Ol10E05	HMR320	2.3	0.0-16.5	E45M54500	E36M61358	111.6	96.4-113.3	0.198	2.6

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hbfphw N04-N13	CB10196b	HMR416a	33.6	24.2-39.6	BRAS039b	E36M5145	93.1	86.9-102.5	0.114	3.6
Hbfphw N05-N14	CB10574	CB10609	42.8	36.8-55.5	E39M61383	Ol12D02	6.0	0.0-13.8	0.109	3.3
Hbfphw N10-N19	E46M59241	E33M54131	17.6	13.3-21.6	E45M54500	E36M61358	103.6	101.5-111.6	-0.101	2.8
Hbfphw N13-N17	Ol10B07	Na10G10a	62.3	58.1-66.3	E43M5158	CB10425	90.0	83.9-94.0	-0.157	6.8
Hbhch N02-N09	E32M54197	E31M5390	11.6	0.0-15.4	Na12E06b	Na14C12	49.5	48.0-53.4	-2.105	2.9
Hbhch N05-N16	CB10545	E33M5048	2.0	0.0-8.0	CB10211b	BRAS048	126.5	120.1-132.5	2.381	3.7
Hbhch N06-N06	E44M60472	E32M48677	8.0	4.0-12.0	E44M60432	E33M60378	50.0	44.0-57.5	4.328	12.2
Hbhch N06-N07	E44M60472	E32M48677	8.0	4.0-12.0	E39M49307	E44M58101	4.0	0.0-16.0	3.814	9.5
Hbhch N08-N10	CB10629	E43M62222	43.1	38.9-49.4	Na12E09	Ol10B11	63.9	50.4-72.2	-3.530	8.1
Hbhch N11-N19	Ol13C10b	E31M55102	29.4	26.5-31.4	E35M62232	MR111c	25.2	21.2-29.3	3.113	6.3
Hbhch N14-N19	E42M5176	CB10493	86.6	80.6-87.4	E34M51252	E32M62386	48.4	46.4-51.2	-2.791	5.1
Hbla N09-N12	E32M48148	E44M51350	3.7	0.0-9.7	E43M62121	HMR353b	61.3	56.8-65.3	-14.25	4
Hbla N12-N12	E44M62128	E32M5072	19.1	14.5-25.1	E32M5183	CB10119	76.2	75.8-84.2	17.07	6

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hby07 N01-N11	E46M6252	E43M51273	39.6	36.8-43.3	E33M50414	Ol13C10b	22.5	10.3-24.5	-0.32	3
Hby07 N03-N19	BRMS043	Na12E02	75.7	72.7-79.7	E43M6282	E33M47135	64.4	60.7-66.4	-0.46	6
Hby07 N04-N18	Na12E05	E45M49114	0.0	0.0-9.3	E32M48253	E37M50120	23.6	13.3-28.9	-0.34	3
Hby07 N09-N11	E33M5080	E31M5395	111.6	106.6-115.6	Ol13C10b	E31M55102	29.4	26.5-31.4	0.38	4
Hby07 N10-N14	E44M60114	E34M62109	0.0	0.0-6.0	HMR363b	Na10C01c	34.4	26.8-37.4	0.27	2
Hby07 N10-N14	E46M59252	Na12E09	56.4	50.4-63.9	HMR363b	Na10C01c	34.4	26.8-37.4	0.44	5
Hby07 N13-N16	E45M59151	E35M62226	43.2	39.0-47.2	E33M49199	Na12E01a	53.3	49.8-57.3	-0.38	4
Hby07 N16-N19	NA12E01a	E34M5445	64.3	58.3-72.5	E32M49386	E45M54500	101.5	94.4-107.6	0.44	5
Hbph07 N01-N19	E32M5451	CB10081	0.0	0.0-5.7	E45M5180	CB10266	10.0	0.0-18.8	-1.12	5
Hbph07 N01-N09	E34M5163	E46M5199	36.5	30.5-38.8	Na10B07	CB10476	100.2	97.6-102.2	-0.89	3
Hbph07 N03-N07	Na14G10	E42M55125	59.2	54.8-63.2	Na12B02	Na12E11	28.6	25.1-32.6	1.04	4
Hbph07 N03-N17	E42M55125	BRMS043	72.7	68.7-74.7	CB10268	E38M49507	58.5	48.9-62.8	1.15	5
Hbph07 N06-N19	E44M60472	E32M48677	8.0	2.0-14.7	Ol12F07	E32M49386	94.4	83.0-103.5	1.33	7

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hbph07 N09-N16	CB10116b	E31M49223	141.9	139.0-145.9	E45M48404	E32M59285	111.7	97.8-122.1	-0.82	3
Hbph07 N13-N17	MR86b	Ol10E05	0.0	0.0-4.3	CB10268	E38M49507	58.5	48.9-62.8	-1.31	7
Hbtsm07 N03-N16	Na14G10	E42M55125	59.2	54.8-63.2	E32M51225	E45M48151	77.9	75.9-85.8	0.05	5
Hbtsm07 N13-N13	Na10G10a	E38M55460	69.9	64.3-71.9	BRAS039b	E36M5145	89.1	84.9-95.1	-0.07	8
Hby06 N01-N17	E33M5465	Na14F11	81.9	76.9-92.5	Ol13C10a	BRAS066b	5.4	0.0-7.4	-0.45	4
Hby06 N06-N07	E44M60472	E32M48677	4.0	0.0-12.0	Na12E11	Ni2D03	43.3	39.3-43.3	-0.43	4
Hby06 N06-N07	E33M60378	Na12D08	55.7	48.0-55.7	Na12E11	Ni2D03	43.3	39.3-43.3	0.41	3
Hby06 N09-N10	CB10116b	E31M49223	145.9	139.0-145.9	Na12E09	Ol10B11	59.9	54.4-65.9	-0.37	3
Hby06 N15-N17	E36M6167	Ol10E04	8.4	5.5-12.4	E35M62246	E36M61110	36.5	32.4-38.5	-0.37	3
Hbtsm06 N02-N14	E36M47496	MR191_2	0.0	0.0-6.9	Na10C01c	HMR416b	39.4	34.4-42.6	-0.021	1.6
Hbtsm06 N03-N08	E44M6255	CB10079	123.6	117.8-129.0	E31M53515	HMR582	24.0	20.8-28.0	0.029	3.2
Hbtsm06 N08-N13	Na12B05	E32M59123	67.4	59.4-76.5	BRAS039b	E36M5145	91.1	81.8-100.5	-0.023	2
Hbtsm06 N09-N16	E42M55131	E31M55249	89.4	83.4-92.5	MR26	E36M47689	19.8	10.0-41.6	0.022	2

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hbtsm06 N10-N13	E39M61362	E46M59252	46.6	42.9-50.4	E33M60156	E45M59151	39.0	36.0-43.0	-0.026	2.6
Hbph06 N02-N13	BRAS031	E32M54197	8.5	0.0-11.6	E38M55460	BRAS068	73.9	71.9-77.8	0.499	2.1
Hbph06 N03-N17	E36M6183	E44M57448	14.5	11.5-20.5	E34M54113	Na12A10	82.6	76.1-87.9	0.721	4.3
Hbph06 N03-N15	E42M55125	BRMS043	70.7	65.2-74.7	CB10374	E36M6167	3.5	0.0-7.5	1.014	8.5
Hbph06 N05-N12	Na12E01b	E31M62195	51.5	47.9-55.5	E44M62128	E32M5072	21.1	12.5-27.1	0.636	3.3
Hbph06 N05-N09	E42M55166	E42M60190	85.5	77.5-87.5	E42M55131	E31M55249	81.4	79.6-87.4	0.890	6.5
Hbph06 N06-N11	E45M49137	E34M55290	68.3	61.5-74.0	E44M50316	E44M4897	86.7	76.7-88.7	-0.605	3
Hbph06 N08-N13	Na12B05	E32M59123	59.4	54.1-63.4	E44M51636	E44M47290	16.5	12.0-20.5	-0.598	2.9
Hbph06 N11-N15	E33M50414	Ol13C10b	22.5	18.8-26.5	Na12C01	CB10027	20.9	18.2-24.9	-0.566	2.6
Hbph06 N11-N13	E31M55102	E32M5452	41.6	37.6-46.5	E33M60156	E45M59151	37.0	34.0-43.0	0.638	3.4
Hbph06 N17-N19	E44M49242	E33M54647	103.0	99.0-107.0	CB10266	E35M62232	14.8	8.0-18.8	-0.475	1.9

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Htdshw N07-N13	E39M49307	E44M58101	0.0	0.0-8.0	E46M59202	E33M60156	36.0	32.0-41.0	0.02	6
Htdphw N03-N08	E36M6183	E44M57448	20.5	11.5-30.6	E33M47216	E46M59160	0.0	0.0-6.0	-0.02	7
Htdphw N11-N16	E43M59241	CB10206	49.2	46.5-53.2	E45M48404	E32M59285	107.7	103.5-111.7	-0.01	5
Htfshw N05-N08	E34M5994	BRAS063	29.5	25.5-31.5	HMR582	E32M51301	32.8	28.0-36.8	0.209	2.6
Htfshw N08-N08	E46M59160	BRAS039a	11.5	8.0-16.0	E31M53515	HMR582	24.0	20.8-26.0	0.500	14.7
Htfshw N11-N17	E44M50316	E44M4897	86.7	76.7-88.7	E44M49242	E33M54647	109.0	107.0-109.0	-0.354	7.4
Htfshw N13-N16	Na10G10a	E38M55460	67.9	64.3-71.9	E32M51225	E45M48151	75.9	68.5-79.8	0.263	4.1
Htfshw N14-N18	Ol10F12	Ol13D02a	15.8	11.8-17.8	E37M5091	E32M51190	9.3	4.0-15.3	0.286	4.8
Htfphw N07-N13	E39M49307	E44M58101	4.0	0.0-12.0	E38M55460	BRAS068	73.9	69.9-75.9	0.129	4.5
Htfphw N13-N17	E35M62226	Ol10B07	52.1	49.2-56.1	E38M49507	Na12H07a	60.8	54.5-64.8	0.133	4.8
Htfphw N13-N17	BRAS039b	E36M5145	93.1	89.1-100.5	E32M4750	E35M62246	34.4	30.4-38.5	-0.200	10.8
Htfphw N14-N19	Ol10F12	Ol13D02a	19.8	17.8-22.8	E45M54500	E36M61358	103.6	99.5-107.6	-0.156	6.5
Hthch N07-N19	Ra2G08	Na12B02	27.1	16.4-32.6	E33M47135	E34M62369	70.4	66.4-74.4	-1.802	5.2

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hthch N08-N10	CB10629	E43M62222	41.1	38.9-45.1	Na12E09	Ol10B11	65.9	59.9-74.2	-2.255	8.2
Htla N01-N09	E33M5465	Na14F11	89.9	83.9-92.5	E46M5987	BRAS020	43.0	37.0-46.0	16.52	6
Htla N05-N15	Na12E01b	E31M62195	51.5	45.9-56.7	CB10374	E36M6167	3.5	2.0-5.5	-13.94	4
Htla N07-N13	E39M49307	E44M58101	4.0	0.0-12.0	E38M55460	BRAS068	75.9	71.9-77.8	16.77	6
Htla N12-N15	E44M62128	E32M5072	25.1	19.1-30.7	CB10027	CB10312	30.3	26.9-34.3	13.33	4
Htslw N01-N05	CB10097	E34M5163	26.4	22.4-32.5	E42M55166	E42M60190	73.5	64.7-79.5	-0.042	5.1
Hty07 N01-N13	E44M60371	CB10097	20.9	10.0-26.4	E33M60156	E45M59151	37.0	34.0-39.0	-0.43	5
Hty07 N03-N16	NA12H06	CB10413	0.0	0.0-3.5	E44M6067	MR26	16.9	8.0-16.9	-0.27	2
Hty07 N03-N18	BRAS056	E43M51108	38.6	30.6-44.6	E33M62150	E36M51251	41.0	36.8-48.7	0.40	5
Hty07 N09-N12	Na10B11	GMR013a	24.8	19.9-28.8	E34M5971	E43M51140	33.6	28.7-41.6	-0.40	5
Hty07 N09-N09	BRAS020	Na12E06b	48.0	43.0-51.5	CB10116b	E31M49223	141.9	137.0-145.9	0.38	4
Hty07 N10-N16	Na12E09	Ol10B11	59.9	52.4-65.9	E44M6067	MR26	16.9	8.0-16.9	0.32	3
Hty07 N12-N19	E45M51177	E43M62121	56.8	50.0-65.3	E33M47256	E44M62104	33.2	29.3-37.2	-0.38	4

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Hty07 N16-N19	E44M6067	MR26	16.9	8.0-16.9	E33M47256	E44M62104	33.2	29.3-37.2	0.28	2
Httsm07 N02-N09	E31M5390	E31M53267	23.4	6.9-29.4	CB10373	Na10B07	97.6	94.5-99.6	0.04	4
Httsm07 N03-N12	BRAS056	E43M51108	36.6	28.6-40.6	E44M62128	E32M5072	25.1	19.1-30.7	0.06	8
Httsm07 N03-N03	Na12E02	CB10036	82.0	77.7-86.0	CB10079	E43M51254	129.0	125.6-135.0	-0.04	4
Htph07 N01-N10	E33M50164	E44M60371	6.0	0.0-12.0	Na12H04	E42M55595	72.2	65.9-76.2	-1.02	5
Htph07 N04-N05	E34M6054	CB10196b	10.2	3.3-20.2	BRAS063	CB10574	34.8	19.5-51.5	0.91	4
Htph07 N08-N13	E32M51301	CB10629	36.9	32.8-38.9	E43M62147	BRMS033	104.2	100.5-106.2	1.15	6
Htph07 N08-N16	E39M47181	Na12B05	54.1	47.4-61.4	MR26	E36M47689	21.8	10.0-33.6	1.05	5
Htph07 N09-N11	CB10116b	E31M49223	141.9	141.0-145.9	E37M59140	CB10587	4.2	0.0-6.2	1.16	7
Htph07 N11-N16	E32M5452	E43M59241	46.5	41.6-53.2	CB10211b	BRAS048	126.5	118.1-132.5	1.03	5
Hty06 N01-N14	E33M50164	E44M60371	10.0	1.7-16.0	CB10196a	E33M62109	52.2	48.6-56.2	0.55	7
Hty06 N05-N10	Na12E01b	E31M62195	51.5	45.9-55.5	E34M62109	E46M59241	13.3	2.0-26.4	-0.48	5
Hty06 N07-N13	E39M49307	E44M58101	0.0	0.0-6.0	Ol10E05	HMR320	4.3	0.0-18.5	0.44	5

QTL	Left flanking marker i	Right flanking marker i	Posit. i (cM)	Range i	Left flanking marker j	Right flanking marker j	Posit. j (cM)	Range j	Effect	Var. contr. (%)
Httsm06 N01-N03	E34M5163	E46M5199	28.5	24.4-32.5	E33M49207	Ra2A06	111.8	105.8-117.8	-0.035	7.8
Httsm06 N01-N13	E44M50235	E33M5465	80.5	70.6-87.9	E38M55460	BRAS068	73.9	71.9-75.9	0.025	3.9
Htph06 N01-N17	E44M4777	E45M57423	56.8	51.6-60.8	Ol13C10a	BRAS066b	3.4	2.0-5.4	-0.715	4.3
Htph06 N06-N10	HMR558	Na12H07b	79.3	76.5-83.3	Na12E09	Ol10B11	59.9	54.4-63.9	-0.618	3.2

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Giessen, early May 2010

ERKLÄRUNG

Erklärung zur Sicherung guter wissenschaftlicher Praxis

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