COMPARATIVE QTL MAPPING IN DIPLOID AND ALLOPLOID *BRASSICA* SPECIES TO ANALYZE FIXED HETEROSIS

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Für meine Eltern

und meine Geschwister

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2 Introduction

2.1 Polyploidy in the evolution of plants

Polyploidy is the occurrence of more than two homologous sets of chromosomes in cells and organisms (Grant 1981). In the evolution of plants polyploidy plays a major role. This is reflected by the large number of species of polyploidy origin (Moody et al. 1993). So the polyploidy level in angiosperms is estimated for a range from 30% to 70% (Stebbins 1950; Masterson 1994). Soltis et al. (2004) stated that there is polyploidy in most organisms somewhere in their evolutionary history. Flowering plants and perhaps all eukaryotes possess genomes with considerable gene redundancy, much of that is likely the result of polyploidy or whole genome duplication. Besides the flowering plants also the majority of vertebrates have descended from polyploid ancestors (Otto 2007). This ancient forms of polyploidy were also defined as paleopolyploids by Tate et al. (2006). Over time these plants may differentiate into distinct species from the normal diploid line. Also most of the agricultural plants are polyploid. Examples for tetraploid crops are durum (*Triticum durum*), maize (*Zea mays*) and potato (*Solanum tuberosum)*. Also hexaploid crops as bread wheat (*Triticum aestivum*) or even octaploid ones as sugar cane (*Saccharum officinarum*) are very common. Parkin et al. (2003) stated that this inherent level of duplication within the genomes of crop species adds an extra level of complexity when attempting to identify regions of homology across species. In defining regions of collinearity between model species and their crop relatives, it is first necessary to define the extent of the genome duplication found within the genome of the crop itself.

Besides the natural occurrence, polyploidy can be induced by using colchicine discovered in 1820 by Pelletier and Caventou which inhibits as a spindle poison the microtubular polymerization during mitosis and so effectively fusions (Lydia and Raja Rao 1982) resulting in cells that contain no chromosome and cells with doubled number of chromosomes.

Two broad categories of polyploids can be recognized, autopolyploids and allopolyploids. Grant (1981) stated that the ‘principal criteria for distinguishing between autopolyploids and amphiploids (allopolyploids) are chromosome behavior, fertility, segregation ratios, and morphology’, and that ‘these criteria will all break down in individual cases.’ He also wrote that autopolyploidy and allopolyploidy are ‘the extreme members of a graded series.’ A strictly

taxonomic approach, with autopolyploidy and allopolyploidy paralleling intraspecific and interspecific polyploidy, respectively, is used by Lewis (1980). Soltis et al. (2004) adhered to this approach, that alloploids form between different species, whereas autoploids form within species, typically involving crossing between individuals. Another criterion to distinguish between autoploids and alloploids is the behavior of chromosome pairing during meiosis. Autopolyploidy is caused by unsuccessful separation of the chromosomes during meiosis. In autopolyploids more than two homologous chromosomes occur in the cells, this means that the similar chromosomes are able to form multivalents and univalents during meiosis.

In contrast the term homoeologous is used to describe the relationship of similar chromosomes or parts of chromosomes brought together following interspecies hybridization and allopolyploidisation. The relationship of the homoeologous chromosomes also was homologous in a common ancestral species. In allopolyploids, the homologous chromosomes within each parental sub-genome should pair faithfully during meiosis, leading to disomic inheritance. In some allopolyploids, the homoeologous chromosomes of the parental genomes may be nearly as similar to one another as the homologous chromosomes. This could lead to tetrasomic inheritance, intergenomic recombinations between the chromosomes, and reduced fertility. Rapid genomic rearrangements, genomic downsizing, movement of genetic elements across genomes, and the movement of foreign genetic materials into the polyploid genome illustrate the complex dynamics of polyploid genomes. Following polyploidization, both genetic and epigenetic mechanisms may play an important role in altering gene expression(Soltis et al. 2004).

2.2 *Brassica* and polyploidy

A very good example for alloploidy is the genus *Brassica* where both diploid and polyploid species exist. The alloplolyploid character of the species *B. carinata*, *B. juncea* and *B. napus* was found by U (1935) investigating the chromosome pairing in crosses between species with high and low chromosome numbers. So he came to the conclusion that *Brassica napus* is an amphidiploid formed of the fusion of two diploid genomes, a C-genome progenitor (*Brassica oleracea*) and an A-genome progenitor (*Brassica rapa*) (U 1935).

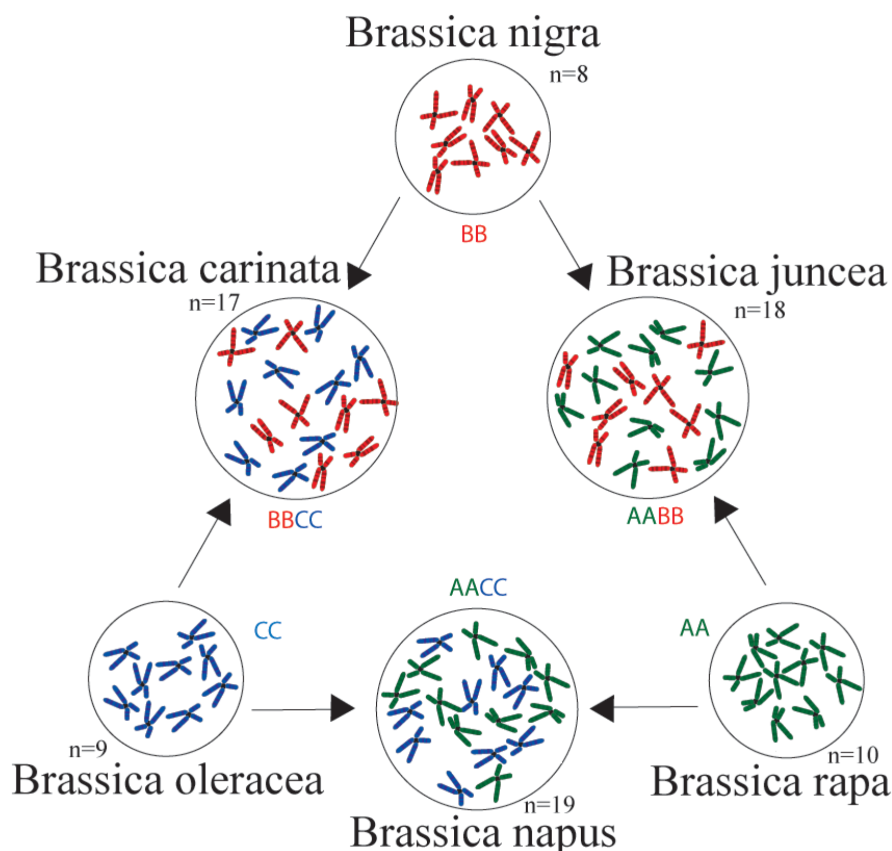


Figure 1: Overview of genetic relationships between various members of the genus *Brassica.* Chromosomes of each of the genomes A, B and C are represented by different colours. The illustration shows the origin of the AABB, AACC and BBCC species which have chromosome sets from their AA, BB and CC ancestors. ('Brassica'. Original work by Mike Jones, for Wikipedia.)

The 'triangle of U' (Figure1) explains how three of the *Brassica* species were derived from three ancestral genomes, denoted by the letters AA, BB, or CC. Also each of these diploid monogenomic *Brassica* species are common crops. The number of chromosomes in each haploid genome is denoted by the letter *n*.

These three diploid *B. oleracea*, *B. rapa* and *B. nigra* exist as separate species. Due to their close relationship interspecific crosses are possible. The interspecific crosses end up in the creation of three new species of tetraploid *Brassica* (Figure 1). Because they contain both diploid genomes derived from the two different ancestral species, these are allotetraploids.

Tetraploids are common in a number of different groups of plants and over time these plants can differentiate into distinct species from the normal diploid line (Soltis et al., 2007). In *Oenothera lamarckiana* the diploid species has 14 chromosomes, this species has spontaneously given rise to plants with 28 chromosomes that have been given the name *Oenothera gigas* (De Vries, 1905). Tetraploids can develop into a breeding population within the diploid population and when hybrids are formed with the diploid population the resulting offspring tend to be sterile triploids, thus effectively stopping the intermixing of genes between the two groups of plants (unless the diploids, in rare cases, produce unreduced gametes).

In molecular studies the genetic relationship between the Brassica species was investigated. So Lagercrantz and Lydiate (1996) stated that there are more homologies between B. rapa and B. oleracea than with B. nigra. This leads to the assumption that the formation of the B. nigra species was earlier than B. rapa and B. oleracea. Recent work in genomics has shown that gene duplications are much more common than thought earlier and not restricted to allopolyploid species. The Brassica species traditionally considered as diploids are actually degenerated polyploids (Lagercrantz 1998) and even in the small Arabidopsis genome gene families are frequently found in tandem replications (The Arabidopsis Genome Initiative 2000).

The genus *Brassica* is remarkable for containing many important agricultural and horticultural crops, most of them annuals or biennials. So in Asia forms of *B. rapa* are used as vegetable and in Europe forms for oil and fodder production are dominating. Also for *B. oleracea* many forms of vegetables are cultivated like cabbage, broccoli, and cauliflower (Song et al. 1990). In contrast rapeseed (*B. napus*) is a relatively new crop (Gruber et al. 2004) and can easily be resynthesized by an interspecific cross followed by chromosome doubling. Such synthetic polyploids are excellent genetic materials for comparative analysis of gene expression and genomic changes in the early stages of polyploid formation because the exact progenitors are known, whereas the

progenitors of many natural allopolyploids are unknown or unavailable (Chen and Ni 2007).

2.3 Fixed heterosis and intergenomic dominance

The wide distribution of polyploidy among plants has led to a variety of theories for the evolutionary advantages of polyploidy. Meyers et al. (2006) claimed that the abundance of polyploidy may be the result of a simple ratcheting process that does not require evolutionary advantages due to the biological properties of organisms and that the evolution of polyploidy is a one-way process in which the number of chromosomes can increase but not decrease.

But there is also the theory that allopolyploids may have an advantage comparable with that of hybrids. Usually the typical chromosome number is doubled in successful allopolyploid species, with four sets of chromosomes the genotypes can form a complete diploid set from the parent species, thus they can produce fertile offspring that can mate and reproduce with each other. Allopolyploidy in plants often results in a benefit in vigour which is comparable to the hybrid vigour as the offspring plants are larger and stronger growing than either of the two parent species. Classically heterosis is defined as the increase in performance of heterozygous F1 plants compared to the mean parental performance (Shull 1948). But in the case of allopolyploids the heterotic effects may occur between homoeologous chromosomes (Figure 2) and therefore will not get lost due to inbreeding. Thus the phenomenon is called fixed heterosis (Soltis et al. 2004).

A: Classical heterosis

B: Fixed heterosis

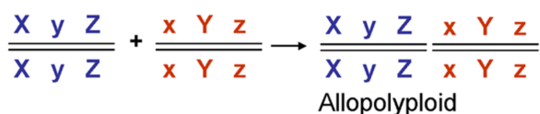


Figure 2: Comparison of classical and fixed heterosis

In a previous project plant material was developed which is suitable to analyse the effects of „fixed" heterosis and polyploidy in the allopolyploid species *Brassica napus*. For this purpose genetically balanced genotypes are needed which vary only in level of ploidy or genome constitution. Therefore starting from a diverse set of DH/Inbredlines of *B. rapa* and *B. oleracea* interspecific hybrids were produced via embryo rescue. A complete set of diploid and tetraploid *B. rapa* and *B. oleracea*, respectively, and their corresponding interspecific allopolyploid hybrids were produced (Abel et al., 2005).

Than this material was used to measure fixed heterosis (Abel 2006). The resynthesized allopolyploid *B. napus* genotypes showed considerable higher fresh matter biomass compared to their parental species. The study showed that the higher performance of resynthesized rapeseed compared to its diploid parental species was based on fixed heterosis and cannot be explained by an effect of the higher ploidy level. Abel (2006) found a magnitude of fixed heterosis averaged 50% relative to the parental mean and 8% relative to the better parent. In contrast the autotetraploid lines yielded from 1% to 64% less than the corresponding diploid lines. The mean midparent classical heterosis was 62% (33% relative to the better parent) in *B. rapa*, 90% (75%) in *B. oleracea* and 20% (4%) in *B. napus*.

Consequently the main advantage of allopolyploidy in evolution might rather be a higher tolerance to inbreeding and the resulting homozygosity than a higher performance in general. This higher tolerance against inbreeding is thereby based on intergenomic heterozygosity and the resulting fixed heterosis. Abel (2006) claimed that fixed heterosis is a decisive factor for the establishment of allopolyploid species.

In the model system *B. napus* it is also possible to analyse dosage effects of whole genomes: the hypothesis is, that already one positive allele in one of the two genomes is sufficient for maximum performance, which means that genotypes of the constitutions like AAAC, AACC and ACCC should have the same performance. This phenomenon can be called "Intergenomic dominance", without such a type of dominance the performance of a genotype with the constitution ACCC should equal the mean of the parents AACC and CCCC (Figure 3).

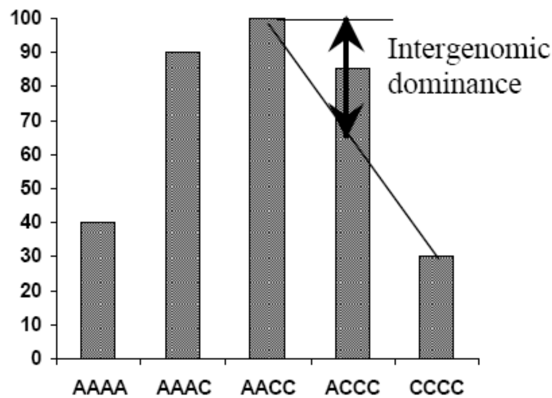


Figure 3: Relative amount of intergenomic dominance

A large number of experiments in quantitative genetics have shown that the main genetic explanation for heterosis is the partial or complete dominance of favourable alleles at many loci. Dominance and epistasis are traditionally considered as different genetic explanations for heterosis, which is of course true from the view of quantitative genetics. For the molecular and physiological understanding of heterosis however, it might be important to distinguish between two types of epistasis. Epistatic interactions may occur between completely different genes, for example genes coding for enzymes involved in different steps within a metabolic pathway, or they may occur between duplicated genes belonging to the same gene family. The biochemical and physiological mechanism of the interactions between two duplicated genes (= epistasis) is probably very similar to the interaction between two different alleles at the same locus (= dominance). For a detailed analysis of fixed heterosis, segregating populations of *B. rapa*, *B. oleracea*, and *B. napus* were developed and QTL for total biomass production were estimated.

The importance of gene duplications is most obvious in allopolyploid species like bread wheat or oilseed rape, where complete genomes are duplicated. *Brassica napus* (genome constitution AACC) is a very suitable experimental system, because it can be artificially resynthesized from the parental species *B. rapa* (AA) and *B. oleracea* (CC) (Becker et al. 1999) .Interactions between genes on different genomes have been called “fixed heterosis” because of their similarity to the “classical” heterosis due to interactions between alleles in heterozygous plants.

Two different questions dealing with duplications in the *Brassica* species and the possible interactions between the genomes have been investigated. The aims of the project were:

(i) to analyze the effect of intergenomic dominance for different dosages of the A and C genome in tetraploids and triploids, and
(ii) to perform a comparative mapping of QTL involved in fixed heterosis between the parental species *B. rapa* and *B. oleracea* and the allopolyploid.

The following chapters 2 and 3 deal with these two questions and try to give an insight into genetic interactions within polyploid species by the use of the *Brassica* family as model plants.

3 Intergenomic dominance

3.1 Introduction

Interspecific hybridization is common in plants and has played a crucial role in the evolution of plant species by generating new ecotypes or new species by allowing gene exchanges across species boundaries. Nowadays, interspecific hybridization is still used in plant breeding to expand the genetic basis of crops and to introduce genes of agronomic importance from wild species into crop germplasm (Leflon et al. 2006). The present interest in dosage effects arises from investigating the phenotypes of transgenic plants differing in copy number of the transgene. Very limited information is available on “dosage effects”: though it is well-known from classical genetics mainly when analysing sex chromosomes in animals (Charlesworth 1996) that the number of copies of a specific allele might be of importance. Such effects have been so far hardly investigated in plants and they are not at all well understood. Cervera et al (2000) found no direct relationship between copy number and expression level and sometimes a higher copy number can even result in reduced expression due to gene silencing(Pickford and Cogoni 2003).

In the model system *B. napus* it is possible to analyse dosage effects of whole genomes: under the hypothesis that already one positive allele in one of the two genomes is sufficient for maximum performance (“intergenomic dominance”) genotypes of the constitutions like AAAC, AACC and ACCC should have the same performance. Not only will the phenomenon intergenomic dominance occur but also heterosis effects, ploidy effects and effects of species hybridization may have an influence.

Within this project the aim was to analyze the effect of intergenomic dominance for different dosages of the A and C genome in tetraploids and triploids. The dosage effects can be analysed alone or in combination with other effects in the used material:

AAAC vs mean of AAAA and AACC: intergenomic dominance for fixed heterosis

ACCC vs mean of CCCC and AACC: intergenomic dominance for fixed heterosis

AAA vs mean of AA and AAAA: effect of ploidy

CCC vs mean of CC and CCCC: effect of ploidy

AAC with AA and AACC: effect of species hybridization

ACC with CC and AACC: effect of species hybridization

AACC vs. mean of AAAA and CCCC: fixed heterosis

mean of AAC and ACC vs mean of AAA and CCC: fixed heterosis

AAC with ACC: additive effects and fixed heterosis

3.2 Material and Methods

3.2.1 Plant Material

The genetic material comprises one *Brassica rapa var. oleifera* line (6748-1430 = A_2A_2) from Agri Food Canada, one *Brassica rapa var. trilocularis* (S2YS-Pb24-2/1 = A_5A_5) from the Genbank Gatersleben and two *Brassica oleracea var. alboglabra* lines (S2C3-4-1 = C_4C_4 and S2Bra165/83-3/1 = C_6C_6) (Crucifer Genet. Cooper. and Genbank Gatersleben). All lines were double haploids or highly inbred lines.

Table 1: *Brassica rapa* (A) und *Brassica oleracea* (C) genotypes used for crossings

Name	Original name	Source [a]	Subspecies	Type[b]
A_2A_2	6748-1430	Agri Food Canada (CA)	*var. oleifera*	DH
C_4C_4	S2C3-4-1	CrGC (USA)	*var. alboglabra*	IL
A_5A_5	S2YS-Pb24-2/1	Genbank Gatersleben (G)	*var. trilocularis*	IL
C_6C_6	S2Bra165/83-3/1	Genbank Gatersleben (G)	*var. alboglabra*	IL

[a] CA: Canada, GB: Great Britain; CrGC: Crucifer Genetic Cooperation; [b] IL: Inbred line, DH: Double haploid

For every used genotype a diploid and a tetraploid type had been developed during former studies (Abel 2005) as well as two resynthesized *Brassica napus* lines ($A_2A_2C_4C_4$, $A_5A_5C_6C_6$).

3.2.2 Crossings

Bud pollinations were made as shown in Figure 4 for the first combination with A_2A_2 and C_4C_4 genotypes and also for the second combination with A_5A_5 and C_6C_6 genotypes. So for both combinations all possible six crossings were performed each.

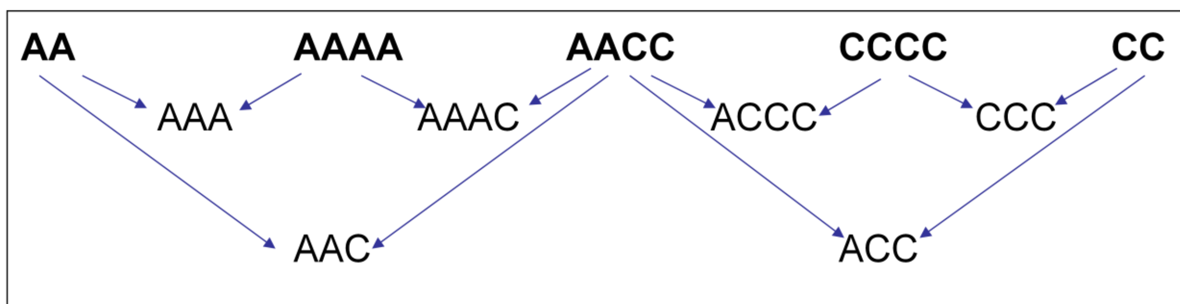


Figure 4: Scheme of all performed crossings, homozygous parental lines are in bold letters, resulting combinations below;

All measured plants were tested with a ploidy analyzer (Partec, Reinach), if the relative amount of DNA corresponded with the expected amount of their ploidy level. Plants with non corresponding results have been excluded from the analysis.

3.2.3 Embryo rescue

To obtain enough data of the AACCxCCCC combinations also plants were developed via embryo rescue of the resulting lines and of both parents. The developing siliques were removed 12 to 14 days after pollination and surface sterilized for 1 minute in 96% alcohol. Under aseptic conditions we dissected the ovules and cultivated them in petridishes on solid medium described by Murashige and Skoog (MS Medium) with 2% saccharose and 1% agarose. Petridishes were breathable sealed and put in a climate chamber under permanent light at 24°C. The developing embryos and plantlets were transferred to new media every third week. By cutting the plants between the internodes and putting the parts on new media for each genotype (AACC, ACCC, CCCC) four clones per four plants were produced at the same size and age. Once roots emerged, the plants were transferred in 7x7 cm pots each, filled with turf soil Fruhstorfer Erde “T 25 (fein)” type.

3.2.4 Biomass trials

After two days of acclimatization in a growth chamber with 15°C, 75% humidity and 16 h of light the plants delivered from embryo rescue were transferred to the greenhouse. The biomass trial consisted of four randomized blocks with four clones each and was surrounded by one row of a standard (Appendix Figure 16). The trial took place in September and October 2007.

The seeds derived from the crossings were germinated in petri dishes on humid filter paper under permanent light at 24°C. After four days the plants were pricked out in four 7x7 cm pots each, filled with turf soil Fruhstorfer Erde “T 25 (fein)” type. A randomized block design with two plants per genotype in four blocks was used (Appendix Figure 15). The trials have been replicated two times during September and October 2007. All blocks have been surrounded by a standard variety to avoid edge effects.

After 33 days after sowing and for the embryo rescue plants after transferring to soil, respectively the total above ground plant biomass was harvested.

Measured traits were the fresh matter (FM) and dry matter (DM). Plant material was dried at 85°C for 48h.

The calculation of least square means was performed with the SAS program version 9.1 (SAS Institute 2003) applying REML under the PROC MIXED procedure.

3.3 Results

3.3.1 Efficiency of Crossings

In total 3651 buds were pollinated in 12 cross combinations (6 in A_2A_2 C_4C_4 and 6 in A_5A_5 C_6C_6) averaging 304.25 buds per combination (Table 2). For all combinations a number of 2158 seeds was produced. The amount of resulting seeds per cross differed from 1240 in the combination $A_2A_2C_4C_4$ x A_2A_2 and only one for A_5A_5 x $A_5A_5A_5A_5$ whereas not all normal looking seeds were able to develop a viable seedling. So of the 31 seeds resulting of the combination $A_2A_2C_4C_4$ x C_4C_4 none resulted in a viable seedling in the greenhouse trials.

Table 2: Efficiency of crossings; Combinations, resulting genotypes, number of hand pollinated buds and the number of the resulting seeds, number in brackets shows number of normal seedlings; ratio between pollinated buds and seeds

Combination	Genotype	No. Pollinated Buds	No. Seeds	Ratio
$A_2A_2C_4C_4$ x C_4C_4	ACC	1312	*31 (0)*	0,024
$A_2A_2C_4C_4$ x $C_4C_4C_4C_4$	ACCC	411	2 (1)	0,005
C_4C_4 x $C_4C_4C_4C_4$	CCC	174	133	0,764
A_2A_2 x $A_2A_2A_2A_2$	AAA	119	20	0,168
$A_2A_2C_4C_4$ x A_2A_2	AAC	245	1240	5,061
$A_2A_2C_4C_4$ x $A_2A_2A_2A_2$	AAAC	230	*15 (0)*	0,065
$A_5A_5C_6C_6$ x C_6C_6	ACC	314	*17 (4)*	0,054
$A_5A_5C_6C_6$ x $C_6C_6C_6C_6$	ACCC	264	53	0,201
C_6C_6 x $C_6C_6C_6C_6$	CCC	140	44	0,314
A_5A_5 x $A_5A_5A_5A_5$	AAA	86	1	0,012
$A_5A_5C_6C_6$ x A_5A_5	AAC	173	588	3,399
$A_5A_5C_6C_6$ x $A_5A_5A_5A_5$	AAAC	413	*14 (1)*	0,034

3.3.2 Combination of tetraploid lines

In all combinations the *Brassica napus* genotype showed the highest amount of plant biomass. In the first combination (Figure 5: A, C, E) the seeds of the ACCC genotype were not viable except one abnormal plant. The embryo rescue plants of that combination grew normal and showed a plant biomass that was significantly higher than the mean of the parental lines.

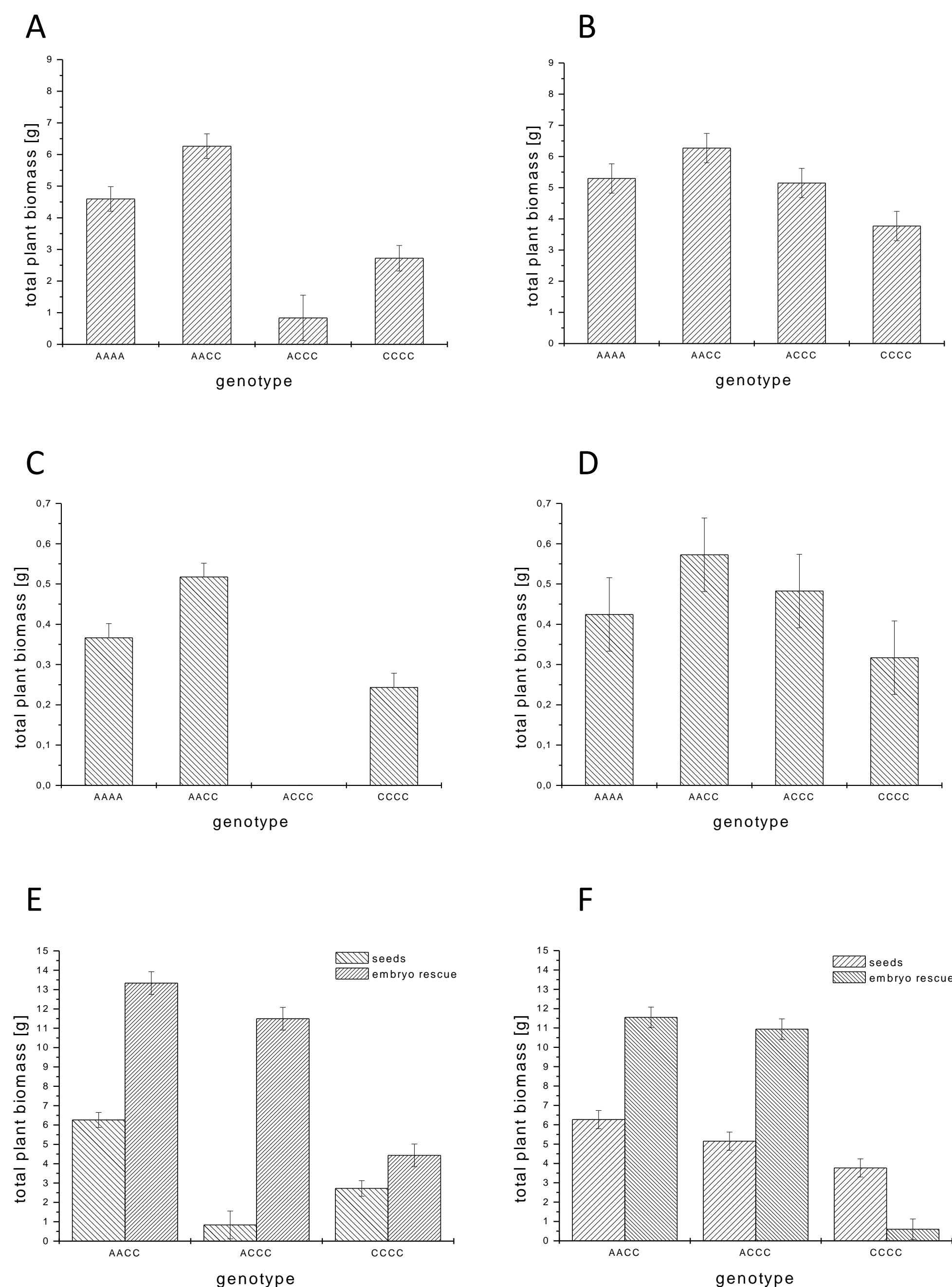


Figure 5: Total plant biomass of the combinations A_2A_2 C_4C_4 (A, C, E) and A_5A_5 C_6C_6 (B, D, F); fresh matter (A, B) and dry matter (C, D); embryo rescue compared with normal sowing (E, F)

The ACCC genotypes of the second combination showed a normal growth in both greenhouse experiments (Figure 5: B, D, F). The tetraploid *Brassica oleracea* derived from embryo rescue were not able to develop normal roots within the media. But in all cases the ACCC performed significantly better than the tetraploid parent and was for dry matter and in the embryo rescue trial not significantly different from the better parent.

In both combinations the ACCC showed a higher plant biomass than the parental mean and the performance was more comparable to the one of the alloploid parent.

The seeds of the AAAC genotypes were all not viable except one in the A_5A_5 C_6C_6 combination that showed an abnormal dwarf-like growth type.

3.3.3 Combination of diploid and tetraploid lines

In the first combination (Figure 6: A, C) the triploid genotypes with only one C genome showed no significant difference from the triploid genotype with no C genome (AAA). In the second combination (Figure 6: B, D) a comparison between AAA and AAC was not possible due to the fact that only one plant with the AAA genotype could be included in the biomass trials. The ACC genotypes of the first combination were all not viable and in the second combination only four out of 16 plants grew and showed a different dry matter content from the other plants. The triploid *oleracea* genotypes showed in both cases a lower biomass than the AAC or ACC genotypes, but this was not significant for the second combination.

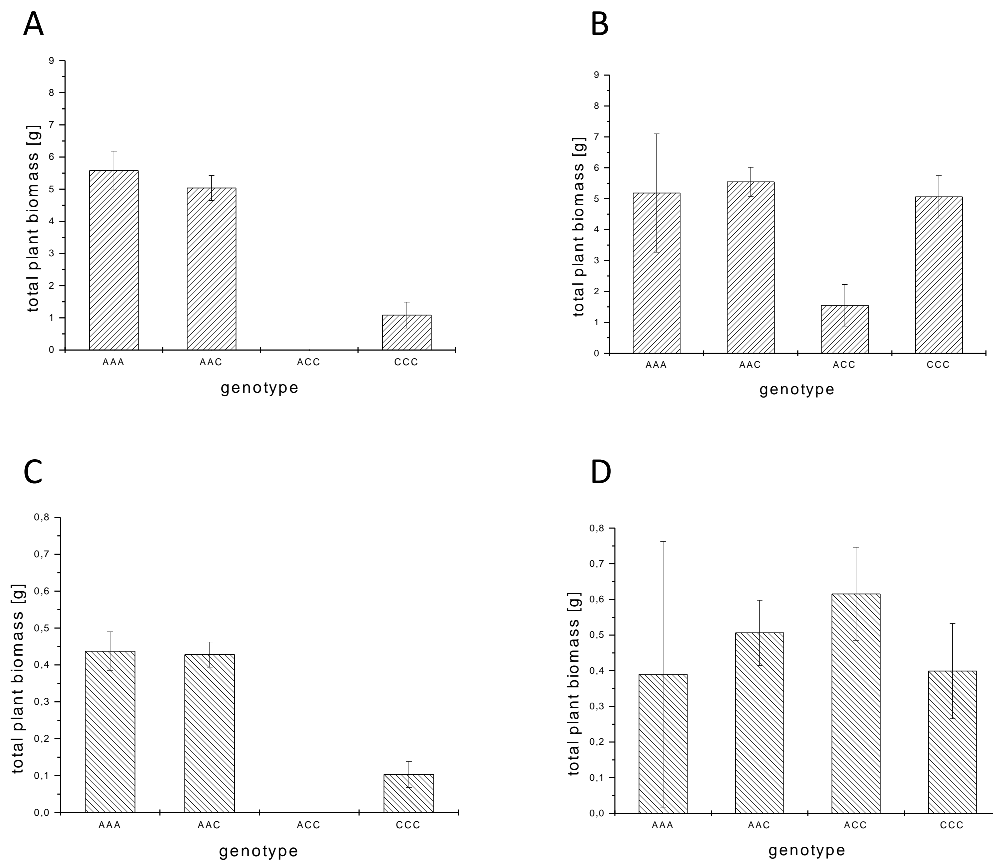


Figure 6: Total plant biomass of the combinations A_2A_2 C_4C_4 (A, C) and A_5A_5 C_6C_6 (B, D); fresh matter (A, B) and dry matter (C, D);

In the A_2A_2 C_4C_4 combinations the triploid AAA showed a higher biomass performance than the diploid and tetraploid parental lines but only the difference with the tetraploid in fresh biomass was significant. The triploid CCC genotype had significantly less fresh biomass and dry matter than both parental lines.

For the second combination the absolute fresh matter values of the triploids AAA and CCC were higher than the ones of the parental diploid and tetraploid genotypes, though not significant. For the dry matter also no significant differences between the different ploidy levels were detected.

3.4 Discussion

The genotypes of the constitution AAAC and ACCC as well as the triploid genotypes will have no normal fertility. Therefore intergenomic dominance was measured for vegetative traits. Early plant biomass is a very fast and reproducible parameter due to the fact that these trials could take place in the greenhouse over the whole year.

In some combinations the bud pollination was not very successful. There the probability of unwanted selfpollination is relatively higher. All resulting plants have been tested with a ploidy analyzer to assure that no self pollination took place. Plants with the amount of DNA as in the parental lines were excluded from the results.

For the genome constitution AAAC 230 and 413 buds were pollinated with a result of only 15 seeds per combination. But none of them germinated. To see if the problems in meiosis depend on the chosen mother plant, we also changed the direction of the pollination. But the results were not influenced by this.

One possibility is that there was not enough time between harvesting the seed and the sowing, so that some of the seed could have been in dormancy. Normally this should not happen because extensive domestication and breeding of crop species have removed most dormancy mechanisms present in the seeds of their wild ancestors, although under adverse environmental conditions, dormancy may appear (Bewley 1997).

The ACCC constitution was for one combination in the seed derived plants and for the other combination in the embryo rescue derived plants significantly better than the tetraploid *B. oleracea* and better than the mean of both parents. For all other cases always one genotype did not show a comparable growth habit. The tetraploid *B. oleracea* of the second combination were not able to build sufficiently long roots on the used medium. Other genotypes or medias should be tested to have comparable results in the plant growth habit.

The results indicate that an intergenomic effect exists because when compared with the tetraploid parental lines a mid parent heterosis effect could be observed in the plants comprising only one copy of the C genome.

Within the triploid forms the AAC genotypes show a biomass yield that was comparable to the better triploid (AAA). The results indicated that intergenomic effects exist and in AAC also heterosis occurs. But the amount of the effect depends directly on the genetic material used for the trials and could not be generalized. Leflon et al. (2006) stated that interspecific crosses contribute significantly to plant evolution enabling gene exchanges between species. The efficiency of interspecific crosses depends on the similarity between the implicated genomes as high levels of genome similarity are required to ensure appropriate chromosome pairing and genetic recombination. Most AAC hybrids from a study of Warwick et al. (2008) had reduced male fertility, intermediate genome structure, and presence of both species-specific amplified fragment length polymorphism markers. Leflon et al. (2006) observed that homologous A chromosomes paired regularly in 71% of the pollen mother cells, and usually one chromosome of each pair was transmitted to the progeny. C chromosomes remained mainly univalent, but were involved in homoeologous pairing in 21.5% of the cells, and 13% of the transmitted C chromosomes were either recombined or broken.

The rate of transmission of C chromosomes depended on the identity of the particular chromosome and on the way the hybrid was crossed, as the male or as the female parent, to *B. napus* or to *B. rapa*.(Leflon et al. 2006). Attia et al. (1987) stated that a high tendency for an allosyndetic pairing between the A and C genomes was expressed by the formation of one or more trivalents in over 50% of PMCs in the two combinations A.AC and AC.A. This demonstrated a high meiotic pairing potential and a small evolutionary difference between the chromosomes of *B*. *campestris* (AA) and *B*. *oleracea* (CC) (Attia et al. 1987) what could explain the possibility of interactions within combined genomes.

(Auger et al. 2005) examined the levels of the same transcripts in hybrid triploid maize that had received unequal genomic contributions, one haploid genome from one parent and two from the other. If allelic expression were merely the additive value in hybrids from the two parents, the midparent values would be observed. In our study the biomass performance of the triploids was better than the midparent values. (Auger et al. 2005) revealed that although transcript levels were clearly nonadditive, transcript levels in triploid hybrids were affected by genomic dosage. To understand the results in our study it would be necessary to investigate the genomic dosage by determine the mRNA expression rate occurring in the different types.

4 Analysis of QTL involved in fixed heterosis

4.1 Introduction

The spontaneous hybridisation of related species by combining their genomes (alloploidy) has played a prominent role in plant evolution, although the mechanisms for the success of allopolyploids are not well understood (Soltis and Soltis 2000; Osborn et al. 2003). A main reason for this success may be the favourable interactions between genes on their homoeologous chromosomes which is similar to the positive interactions between different alleles causing heterosis in heterozygous genotypes. Those favourable interactions between homoeologous loci should result in an increased performance of allopolyploids compared to their parental species, even in homozygous genotypes (Figure 2). Therefore, such positive epistatic interactions can be called “fixed heterosis” (Abel et al. 2005).

Quantitative trait loci (QTL) are since a few years very often used to get an insight in complex traits (Kearsey and Farquhar 1998). Analytical methods locate QTL with poor precision (10-30 cM), unless the heritability of an individual QTL is high. But besides this many QTL map close to candidate genes, and there is growing evidence from synteny studies of corresponding chromosome regions carrying similar QTL in different species (Osborn et al. 1997).

Brassica napus (genome constitution AACC) is a very suitable model system to analyse “fixed” heterosis via QTL-mapping, because artificial “resynthesized” lines can easily be developed from diploid parental species *B. rapa* (AA) and *B. oleracea* (CC). Fixed heterosis is not depending on heterozygosity, and therefore the mapping populations consist of homozygous plants (instead of testcrosses required to analyse QTL for classical heterosis).

The aim of this project was to perform a comparative mapping of QTL involved in fixed heterosis between the parental species *B. rapa* and *B. oleracea* and the allopolyploid.

4.2 Materials and Methods

4.2.1 Materials

4.2.1.1 Plant Materials

The genetic materials comprise one *Brassica rapa var. trilocularis* line (RO18 = A_1A_1) from the John Innes Center, one *Brassica rapa var. oleifera* line (6748-1430 = A_2A_2) from Agri Food Canada and two *Brassica oleracea var. alboglabra* lines (A12 = C_3C_3 and S2C3-4-1 = C_4C_4) (John Innes Center and Crucifer Genet. Cooper.) (Table 3). All lines were double haploids or highly inbred lines. These four lines have been selected, because the two resynthesized genotypes produced from them, $A_1A_1C_3C_3$ and $A_2A_2C_4C_4$, showed a high amount of fixed mid-parent-heterosis between 49.9% and 70.5% (Abel et al. 2005). To identify the contribution of individual genes to the fixed heterosis by QTL mapping, three segregating recombinant inbred line (RIL) populations were developed: two in the diploid parental species only segregating for loci in the A and C genome (A_1A_2, C_3C_4), respectively, and a third one developed from a corresponding allopolyploid ($A_1A_2C_3C_4$) which is segregating for loci in both genomes. Therefor the genotypes A_1A_1 and A_2A_2, C_3C_3 and C_4C_4 and the two resynthesized *Brassica napus* $A_1A_1C_3C_3$ and $A_2A_2C_4C_4$ were crossed and the F_1 plants were selfed. Via single seed descent up to F_5/F_6 for the diploid species a sample of 150 RILs each and in the allopolyploid 222 RILs were developed.

Table 3: *Brassica rapa* (A) und *Brassica oleracea* (C) genotypes used for mapping of fixed heterosis QTL

Name	Original name	Source [a]	Subspecies	Type [b]
A_1A_1	RO18	John Innes Center (GB)	*var. trilocularis*	IL
A_2A_2	6748-1430	Agri Food Canada (CA)	*var. oleifera*	DH
C_3C_3	A12	John Innes Center (GB)	*var. alboglabra*	DH
C_4C_4	S2C3-4-1	CrGC (USA)	*var. alboglabra*	IL

[a] CA: Canada, GB: Great Britain; CrGC: Crucifer Genetic Cooperation; [b] IL: Inbred line, DH: Double haploid

4.2.2 Methods

4.2.2.1 Biomass trials

Phenotypic data for the QTL analysis of fixed heterosis were evaluated via early biomass measurement of all genotyped RILs. The plants were grown under controlled greenhouse conditions in an alpha lattice design that was constructed with Plabplan (Utz 1994). Each of the 37 incomplete plots consisted of six lines, eight plants per RIL, planted in four 7x7 cm pots each and filled with turf soil Fruhstorfer Erde "T 25 (fein)" type. The trials have been replicated four times from April 2008 till June 2008. The harvest of total plant biomass was 18 and 22 days after sowing. Measured traits were the fresh matter (FM) and dry matter (DM) at both harvest times and the absolute growth between both harvest times. Plant material was dried at 85°C for 48h.

The adjusted means of the each replicate were calculated with SAS program version 9.1 (SAS (Institute 2003) applying PROC GLM procedure under consideration of the number of germinated plants per pot. Analysis of variances and correlations of the phenotypic data were calculated with Plabstat (Utz 2004) using the LATTICE procedure. The statistical model for a lattice design implemented in PLABSTAT is:

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk},$$

where Y_{ijk} is an observation of genotype k in block j of a replication i; μ is the general mean; r_i is the effect of replication i; b_{ij} is the effect of block j in replication i; g_k is the effect of genotype k; e_{ijk} is the error of observation Y_{ijk}.

4.2.2.2 Total DNA Extraction

The extraction of the DNA was performed with Nucleon©PhytoPure© extraction kits (GE Healthcare) using the mini-prep kits for 0.1g of fresh or deep frozen plant material according to the instructions of the manual provided with the kit. The DNA concentration was measured with a Bio-Rad VersaFluor™ Fluorometer (Bio-Rad, CA, USA) according to the manufacturer's manual using Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad, CA, USA).

4.2.2.3 Marker Analyis

For the construction of the framework maps 28 amplified fragment length polymorphism (AFLP) primer pairs were used (s). The EcoRI primers used in AFLP analysis were labeled with one of the following four fluorescent dyes: (6, 5)FAM, NED, VIC, or PET (Applied Biosystems, Darmstadt, Germany). AFLP analyses were carried out following the protocol of Vos *et al.* (1995) modified for multiplexing in the PCR according to F. Kopisch-Obuch (personal communication): 250 ng DNA were digested in 30 µl RL buffer (10 mM Tris-Acetate, 10 mM Mg-Acetate, 50 mM K-Acetate, 5 mM DTT, pH 7.5) with 4 U EcoRI (Fermentas, St.Leon-Rot, Germany) and 4 U MseI (New England Biolabs, Frankfurt, Germany) for 1.5 h at 37°C. After adding 10 µl of a mix containing 5 pmol EcoRI adapter, 50 pmol MseI adapter, 1 mM ATP and 1 U T4 DNA ligase (Promega, Mannheim, Germany) in RL buffer, DNA and adapters were ligated in a time series of different temperatures (3 h 10 min 37°C, 3 min 33.5°C, 3 min 30°C, 4 min 26°C and finally 15 min 22° C). The final restriction-ligation product (RL) was diluted 1:5 with HPLC grade water. For preamplification 8 µl of the diluted RL were added to 12 µl of a reaction mix giving final concentrations of 1x Taq buffer (Solis Biodyne, Tartu, Estonia, Reaction buffer B), 3.125 mM $MgCl_2$, 0.45 mM dNTPs, 10 pmol EcoRI+1 primer, 9 pmol MseI+1 primer and 2.5 U Taq DNA polymerase (FIREPol, Solis Biodyne). The preamplification was carried out in a Biometra T1 Thermocycler (Biometra GmbH, Göttingen, Germany) with the following program: 94°C for 30s, 20 cycles of 94°C for 30s, 56°C for 30s and 72°C for 2 min, and a final 5 min at 72°C. The preamplification product was diluted 1:10 with HPLC grade water. The final AFLP amplification used 6 µl of the diluted preamplification product in a total reaction volume of 20 µl containing 1x Taq buffer, 0.36 mM dNTPs, 3.125 mM $MgCl_2$, 1 U Taq polymerase, 7 pmol MseI+3 primer, 2 pmol of (6, 5)FAM labeled EcoRI+3 primer, 2 pmol of VIC labeled EcoRI+3 primer, 4 pmol of NED labeled EcoRI+3 primer, and 6 pmol of PET labeled EcoRI+3 primer. The protocol for the Thermocycler was as follows: 1 cycle of 94°C for 1 min, 65°C for 30s, and 72°C for 2 min, 12 cycles of 94°C for 30s, 64.2°C for 30s and 72°C for 2 min, 25 cycles of 94°C for 30s, 56°C for 30s and 72°C for 2 min, and finally 72°C for 5 min.

The AFLP products were separated on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using 50 cm capillary arrays and GeneScan-500 LIZ size standard (Applied Biosystems). GeneMapper v3.7 software (Applied Biosystems) was used for a semi-automatic marker scoring. Since in GeneMapper v3.7's output AFLP primer combinations are written as markers and the actual AFLP markers as alleles of these markers a Perl script,

'Extract_marker', was developed to transform GeneMapper's output into a marker matrix.

To allow an alignment to other maps in the literature 40 simple sequence repeat (SSR) markers covering each of the *B. napus* linkage groups were mapped. SSR marker analyses were performed as described by (Ofori et al. 2008) following the M13-tailing PCR technique (Schuelke 2000). The M13-universal primer was labeled with the fluorophores 6-carboxy-fluorescine (6FAM™), hexachloro-6-carboxy-fluorescine (HEX™), NED™ and PET™. This provided the possibility of simultaneously loading a mixture of 4 differently labeled PCR products which were also analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using 50 cm capillary arrays and GeneScan-500 LIZ size standard (Applied Biosystems) the same way as for the AFLP markers.

For the raw data analysis the software Gene Mapper v. 3.0 (Applied Biosystems) has been used for both AFLP and SSR analysis. A framework map has been constructed using MAPMAKER/EXP 3.0 (Lincoln et al. 1995). The LOD was set at 5.0 and the maximum genetic distance was 35cM. The cM distance between two linked loci was calculated from the recombination frequences after the algorithm of Kosambi (1944).The creation of the maps took place as described by Radoev (2007)

The markers were tested for a independent segregation via Pearson's chi-square index. All markers have been scored for double cross-over. Occurred irregularities have been controlled in the raw data.

4.2.2.4 QTL Mapping

QTL analyses were performed with the software QTLMAPPER 1.6 (Wang et al. 1999). The program allows simultaneous interval mapping of both main effect and digenic epistatic QTL in RIL. First in a stepwise regression of the whole genome markers with significant influence - so called cofactors - were identified. The applied threshold probability was P = 0.01 for the main effect QTL and 0.005 for the epistatic QTL. Via composite interval mapping in a second step the identified genomic regions were tested. The applied threshold probability was also P = 0.01 for main effect QTL and P = 0.005 for epistatic QTL. Finally genetic parameters and effects were calculated for the main effect and epistatic QTL that showed a higher LOD score than the applied thresholds.

The software R/qtl (Broman et al. 2003) was used to draw graphs that show the LOD-scores over the whole chromosomes for all six examined traits.

4.2.2.5 Principles of QTL mapping in allopolyploids

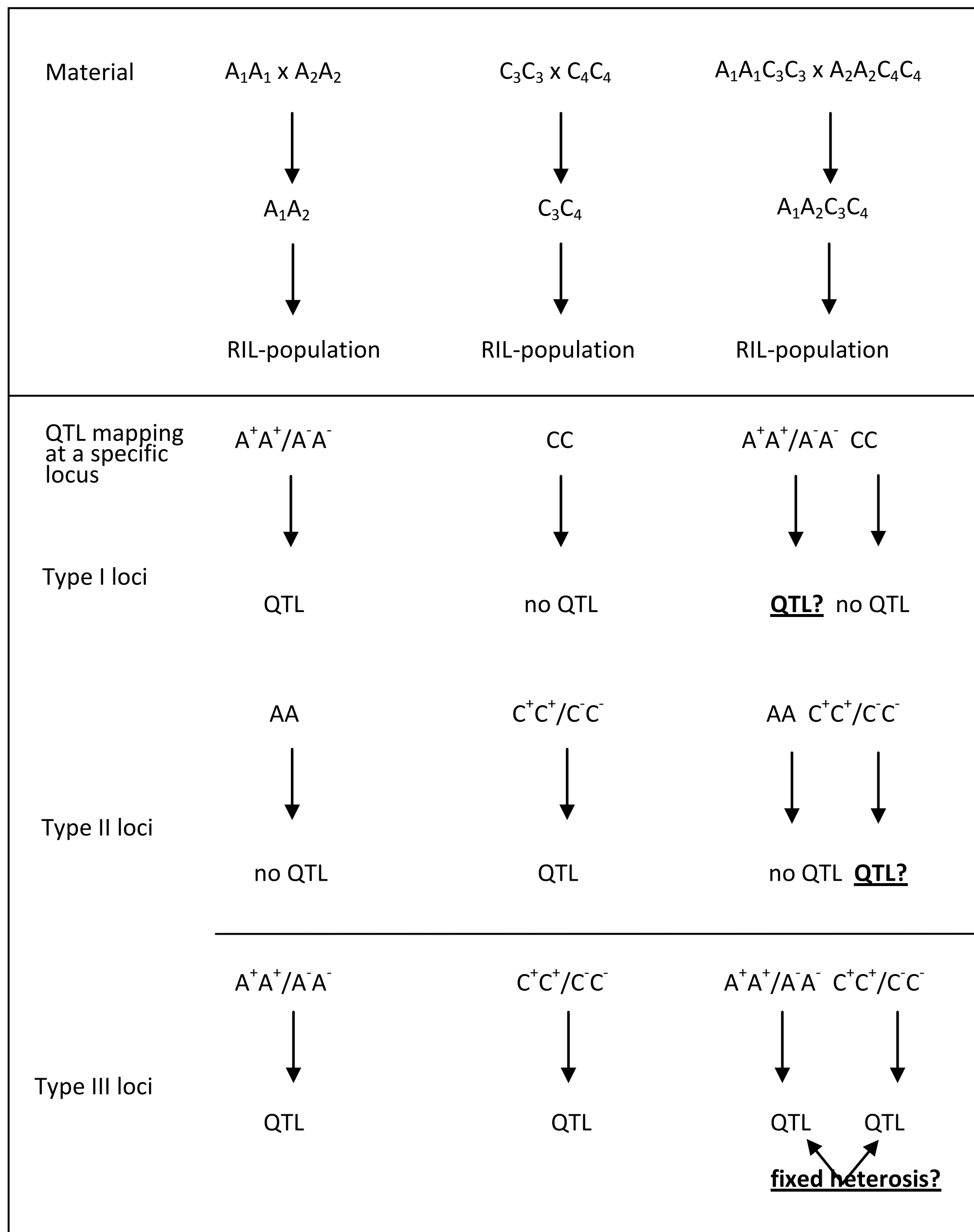


Figure 7: Principles of QTL mapping to analyse fixed heterosis (for explanation see text)

For the observed QTL three types can be classified as follows (Figure 7): QTL which are detected in the A genome but not at the corresponding position in the C genome (I). QTL which are detected in the C genome but not at the corresponding position in the A genome (II) and QTL which are detected both in the A and C genome at corresponding positions (III). The principle of the QTL mapping is the comparison of QTL identified in the A and C genome alone with the same QTL in the allopolyploid situation. Under the assumption of fixed

heterosis the effects of QTL are expected to be different in the allopolyploid situation. At least some of the QTL only detected in the A genome (I) should disappear in the allopolyploid, because the C genome may contain a positive allele which is masking the effect in the other genome. Likewise some of the QTL only detected in the C genome (II) should disappear, because the A genome may contain a positive allele. QTL detected in both genomes (III) will not disappear, but there should be an epistatic effect between them, because ($A^+A^+C^-C^-$ + $A^-A^-C^+C^+$) should have a higher performance than ($A^+A^+C^+C^+$ + $A^-A^-C^-$ C). The assumption is only valid if the effect of a negative allele in one genome can be completely compensated by a positive allele in the other; otherwise the QTL will not completely disappear but will have a smaller effect. For the interpretation of all three types an alignment is necessary to identify the corresponding positions.

4.3 Results

4.3.1 Results of the biomass trials

A significant difference at a 0.01 or 0.05 level of probability was observed between the different genotypes in the three populations for all measured traits (Table 4) besides DM 2 – DM 1 in C_3C_4. Compared to the mean of the A_1A_2 and C_3C_4 lines, the mean of $A_1A_2C_3C_4$ showed a higher biomass yield for both harvest times and for fresh and dry matter each (Table 4).

Table 4: Adjusted means, least significant deviation at 5% (LSD), extreme values and the results of the analysis of variance for the measured traits in the three RIL populations.

Trait	Population	Mean	LSD	Min	Max	Var.comp.	F-value
FM 1	A_1A_2	1.036[a]	0.394	0.309	1.901	0.0487	3.25 **
	C_3C_4	0.836[b]	0.239	0.420	1.280	0.0179	3.42 **
	$A_1A_2C_3C_4$	1.400[c]	0.572	0.267	2.283	0.1313	4.10 **
DM 1	A_1A_2	0.071[a]	0.028	0.017	0.131	0.0002	2.96 **
	C_3C_4	0.058[a]	0.019	0.026	0.089	0.0001	2.63 **
	$A_1A_2C_3C_4$	0.093[b]	0.041	0.017	0.162	0.0006	3.86 **
FM 2	A_1A_2	2.296[a]	0.665	0.869	3.680	0.2452	5.10 **
	C_3C_4	1.685[b]	0.407	1.050	2.482	0.0596	3.79 **
	$A_1A_2C_3C_4$	2.963[c]	1.104	0.856	5.11	0.4143	3.62 **
DM 2	A_1A_2	0.166[a]	0.049	0.053	0.264	0.0012	4.82 **
	C_3C_4	0.126[b]	0.034	0.068	0.191	0.0004	3.65 **
	$A_1A_2C_3C_4$	0.202[c]	0.079	0.063	0.371	0.0023	3.79 **
FM 2 - FM 1	A_1A_2	1.259[a]	0.706	0.482	2.161	0.0852	2.20 **
	C_3C_4	0.849[b]	0.419	0.340	1.596	0.0129	1.57 **
	$A_1A_2C_3C_4$	1.566[c]	1.129	0.321	2.889	0.0713	1.43 **
DM 2 - DM 1	A_1A_2	0.094[a]	0.051	0.028	0.163	0.0005	2.35 **
	C_3C_4	0.068[b]	0.035	0.016	0.130	0.0001	1.87 **
	$A_1A_2C_3C_4$	0.109[a]	0.082	0.015	0.224	0.0005	1.51 **

** Significance at 0.01 level of probability, * significance at 0.05 level of probability and + significance at 0.10 level of probability, respectively; [abc] numbers with different letters are significantly different

Dry matter and fresh matter weight of the same harvest times are highly correlated in all RIL populations (Table 5). Also for different harvest times the traits were correlated between 0.72 and 0.81.

Table 5: Correlations between dry matter and fresh matter

Population	Trait	FM 1	DM 1	FM 2
A_1A_2				
	DM 1	0.95 **		
	FM 2	0.79 **	0.73 **	
	DM 2	0.73 **	0.72 **	0.96 **
C_3C_4				
	DM 1	0.95 **		
	FM 2	0.78 **	0.72 **	
	DM 2	0.73 **	0.72 **	0.95 **
$A_1A_2C_3C_4$				
	DM 1	0.97 **		
	FM 2	0.81 **	0.78 **	
	DM 2	0.80 **	0.80 **	0.97 **

** Significance at 0.01 level of probability, * significance at 0.05 level of probability and + significance at 0.10 level of probability, respectively

The dry matter content at the first harvest date was 6.7 % for A_1A_2, 7.1 % for C_3C_4 and 7.1 % for $A_1A_2C_3C_4$. At the second harvest date the dry matter content for A_1A_2 was 7.4 %, for C_3C_4 7.7 % and 7.1 % for $A_1A_2C_3C_4$.

For all traits the relative midparent heterosis between the performance of the A_1A_2 and C_3C_4 populations and the $A_1A_2C_3C_4$ population was calculated (Table 6). The highest amount of heterosis was observed in FM1 with 48.9%. All traits dealing with dry matter showed an over 10% lower amount of mid parent heterosis.

Table 6: Relative midparent heterosis (rel. MPH) in % for the analyzed traits

Trait	Rel. MPH
FM 1	48.9
DM 1	38.5
FM 2	48.4
DM 2	33.3
FM 2- FM 1	48.8
DM 2- DM 1	37.5

4.3.2 Marker Screening and Construction of the Genetic Map

From the 99 screened AFLP combinations the 28 primer pairs with the most clearly scorable banding patterns and showing most polymorphism between the parental lines have been selected for marker analyses in all three RIL populations.

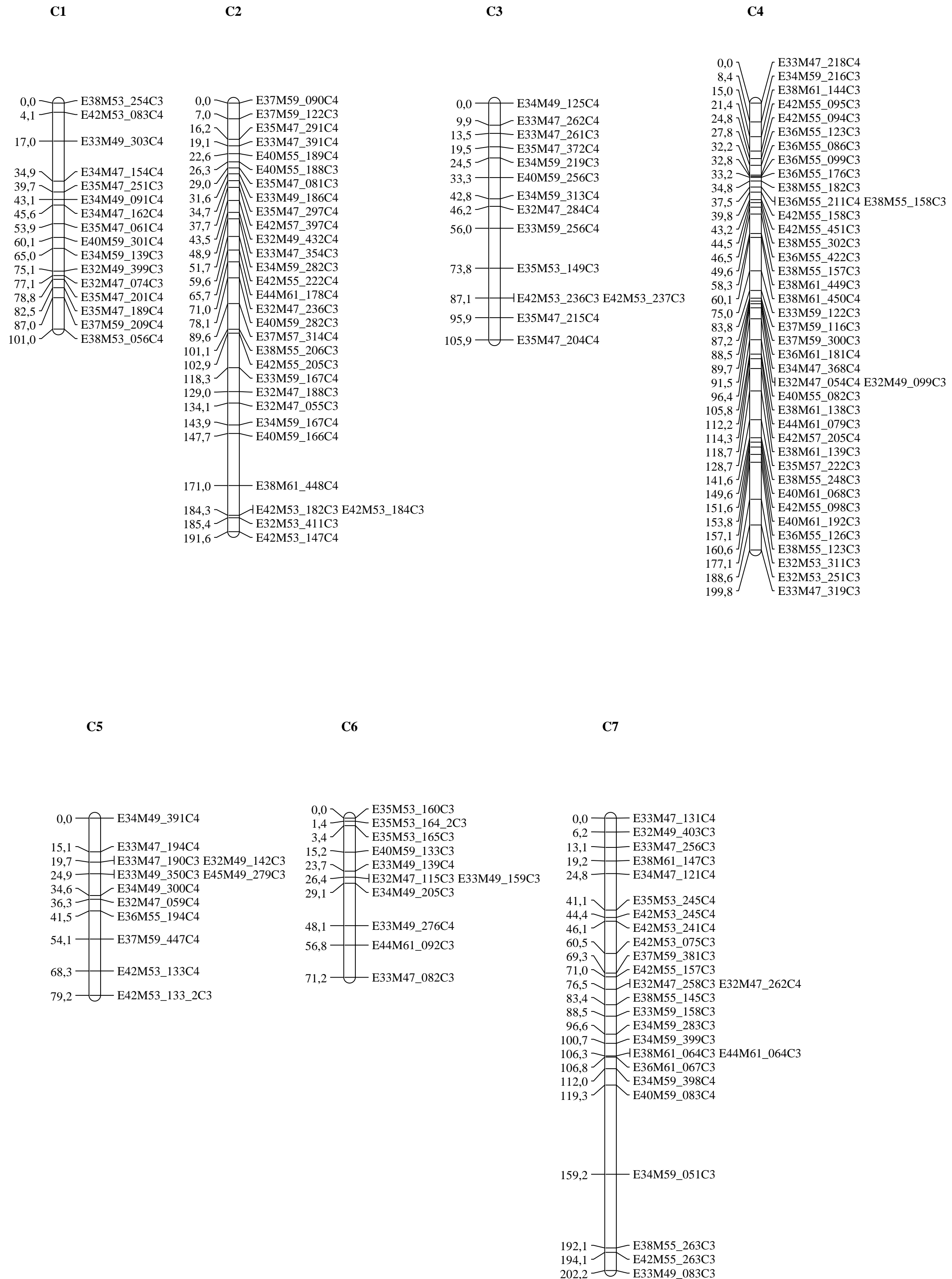
Within the marker screening in GeneMapper for the AA population 372 heterozygous markers have been detected, 279 of them were informative and could be used in the mapping. For the CC population from 400 polymorphic markers 283 were used for the mapping.

For the allopolyploid population 587 markers have been detected from which 137 showed a skewed segregation. In a first step all markers that showed a normal chi square distribution were mapped in a primary map. Then the remaining primer pairs and the ones that showed skewed segregation were added using the try command in MapMaker. Five markers were removed because they acted as crosslinkers between different linkage groups. Finally 276 markers could be placed in the map.

The screening of the SSR primer pairs resulted in 40 markers that detected a single locus or a polymorphic locus for the parental resynthesized lines. Twenty SSR were positioned in the linkage map to align the resulting linkage groups with the map of Radoev et al. (2008).

The derived genetic map for the *Brassica rapa* population consists of ten linkage groups and has a lentgh of 1081 cM (Figure 9). The genetic map for the *Brassica oleracea* population consists of 15 linkage groups with a total length of 1509.1 cM (Figure 8). For the allopolyploid mapping population the genetic map has a length of 2373.4 cM on 23 linkage groups (Figure 10).

For the construction of the framework map for the QTL analyses the most evenly distributed markers were used and in case of very closely linked markers the more informative one remained. In the final map also all SSR had been removed, because they were tested in a subpopulation of 96 RIL to allow an alignment and identification of the corresponding linkage group.


C1
0,0 E38M53_254C3
4,1 E42M53_083C4
17,0 E33M49_303C4
34,9 E34M47_154C4
39,7 E35M47_251C3
43,1 E34M49_091C4
45,6 E34M47_162C4
53,9 E35M47_061C4
60,1 E40M59_301C4
65,0 E34M59_139C3
75,1 E32M49_399C3
77,1 E32M47_074C3
78,8 E35M47_201C4
82,5 E35M47_189C4
87,0 E37M59_209C4
101,0 E38M53_056C4
C2
0,0 E37M59_090C4
7,0 E37M59_122C3
16,2 E35M47_291C4
19,1 E33M47_391C4
22,6 E40M55_189C4
26,3 E40M55_188C3
29,0 E35M47_081C3
31,6 E33M49_186C4
34,7 E35M47_297C4
37,7 E42M57_397C4
43,5 E32M49_432C4
48,9 E33M47_354C3
51,7 E34M59_282C3
59,6 E42M55_222C4
65,7 E44M61_178C4
71,0 E32M47_236C3
78,1 E40M59_282C3
89,6 E37M57_314C4
101,1 E38M55_206C3
102,9 E42M55_205C3
118,3 E33M59_167C4
129,0 E32M47_188C3
134,1 E32M47_055C3
143,9 E34M59_167C4
147,7 E40M59_166C4
171,0 E38M61_448C4
184,3 E42M53_182C3 E42M53_184C3
185,4 E32M53_411C3
191,6 E42M53_147C4
C3
0,0 E34M49_125C4
9,9 E33M47_262C4
13,5 E33M47_261C3
19,5 E35M47_372C4
24,5 E34M59_219C3
33,3 E40M59_256C3
42,8 E34M59_313C4
46,2 E32M47_284C4
56,0 E33M59_256C4
73,8 E35M53_149C3
87,1 E42M53_236C3 E42M53_237C3
95,9 E35M47_215C4
105,9 E35M47_204C4
C4
0,0 E33M47_218C4
8,4 E34M59_216C3
15,0 E38M61_144C3
21,4 E42M55_095C3
24,8 E42M55_094C3
27,8 E36M55_123C3
32,2 E36M55_086C3
32,8 E36M55_099C3
33,2 E36M55_176C3
34,8 E38M55_182C3
37,5 E36M55_211C4 E38M55_158C3
39,8 E42M55_158C3
43,2 E42M55_451C3
44,5 E38M55_302C3
46,5 E36M55_422C3
49,6 E38M55_157C3
58,3 E38M61_449C3
60,1 E38M61_450C4
75,0 E33M59_122C3
83,8 E37M59_116C3
87,2 E37M59_300C3
88,5 E36M61_181C4
89,7 E34M47_368C4
91,5 E32M47_054C4 E32M49_099C3
96,4 E40M55_082C3
105,8 E38M61_138C3
112,2 E44M61_079C3
114,3 E42M57_205C4
118,7 E38M61_139C3
128,7 E35M57_222C3
141,6 E38M55_248C3
149,6 E40M61_068C3
151,6 E42M55_098C3
153,8 E40M61_192C3
157,1 E36M55_126C3
160,6 E38M55_123C3
177,1 E32M53_311C3
188,6 E32M53_251C3
199,8 E33M47_319C3
C5
0,0 E34M49_391C4
15,1 E33M47_194C4
19,7 E33M47_190C3 E32M49_142C3
24,9 E33M49_350C3 E45M49_279C3
34,6 E34M49_300C4
36,3 E32M47_059C4
41,5 E36M55_194C4
54,1 E37M59_447C4
68,3 E42M53_133C4
79,2 E42M53_133_2C3
C6
0,0 E35M53_160C3
1,4 E35M53_164_2C3
3,4 E35M53_165C3
15,2 E40M59_133C3
23,7 E33M49_139C4
26,4 E32M47_115C3 E33M49_159C3
29,1 E34M49_205C3
48,1 E33M49_276C4
56,8 E44M61_092C3
71,2 E33M47_082C3
C7
0,0 E33M47_131C4
6,2 E32M49_403C3
13,1 E33M47_256C3
19,2 E38M61_147C3
24,8 E34M47_121C4
41,1 E35M53_245C4
44,4 E42M53_245C4
46,1 E42M53_241C4
60,5 E42M53_075C3
69,3 E37M59_381C3
71,0 E42M55_157C3
76,5 E32M47_258C3 E32M47_262C4
83,4 E38M55_145C3
88,5 E33M59_158C3
96,6 E34M59_283C3
100,7 E34M59_399C3
106,3 E38M61_064C3 E44M61_064C3
106,8 E36M61_067C3
112,0 E34M59_398C4
119,3 E40M59_083C4
159,2 E34M59_051C3
192,1 E38M55_263C3
194,1 E42M55_263C3
202,2 E33M49_083C3

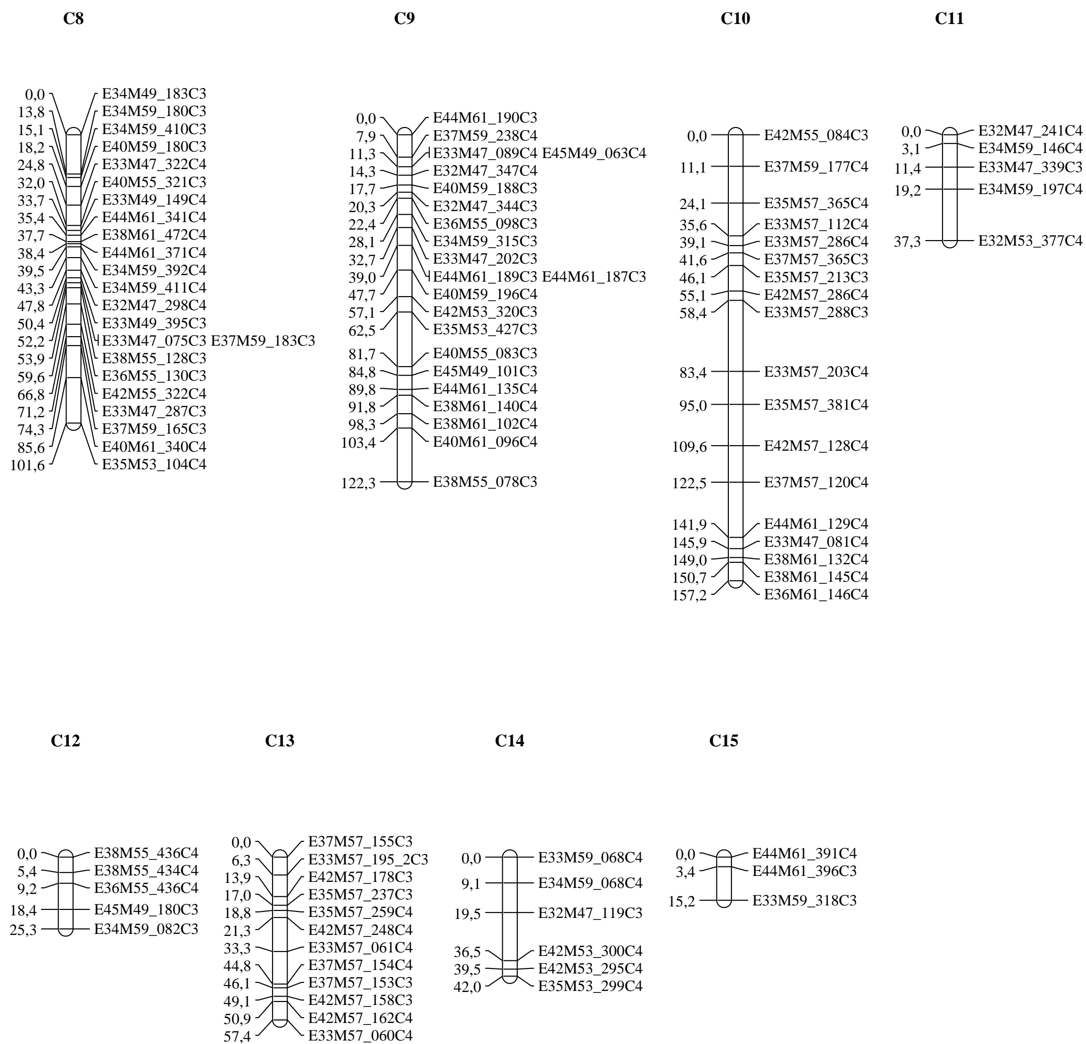
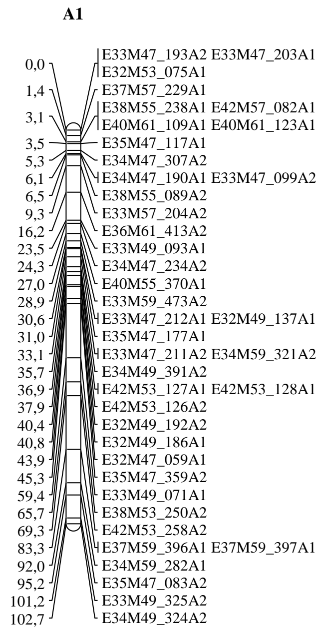
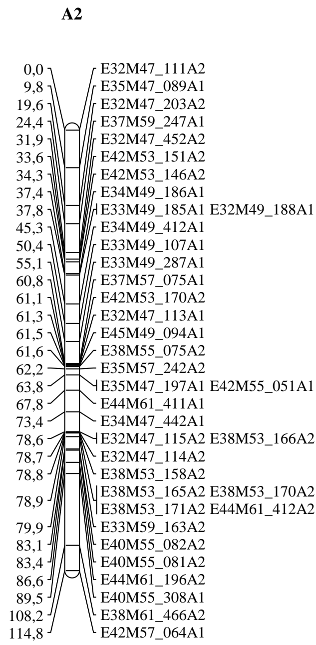
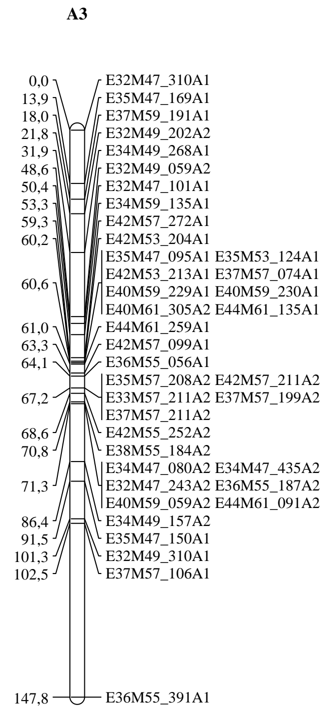


Figure 8: Genetic linkage map of *Brassica oleracea* (cross C_3C_4): Marker loci are presented in absolute positions from the beginning of the linkage groups in cM.


A1
0,0 E33M47_193A2 E33M47_203A1
E32M53_075A1
1,4 E37M57_229A1
3,1 E38M55_238A1 E42M57_082A1
E40M61_109A1 E40M61_123A1
3,5 E35M47_117A1
5,3 E34M47_307A2
6,1 E34M47_190A1 E33M47_099A2
6,5 E38M55_089A2
9,3 E33M57_204A2
16,2 E36M61_413A2
23,5 E33M49_093A1
24,3 E34M47_234A2
27,0 E40M55_370A1
28,9 E33M59_473A2
30,6 E33M47_212A1 E32M49_137A1
31,0 E35M47_177A1
33,1 E33M47_211A2 E34M59_321A2
35,7 E34M49_391A2
36,9 E42M53_127A1 E42M53_128A1
37,9 E42M53_126A2
40,4 E32M49_192A2
40,8 E32M49_186A1
43,9 E32M47_059A1
45,3 E35M47_359A2
59,4 E33M49_071A1
65,7 E38M53_250A2
69,3 E42M53_258A2
83,3 E37M59_396A1 E37M59_397A1
92,0 E34M59_282A1
95,2 E35M47_083A2
101,2 E33M49_325A2
102,7 E34M49_324A2


A2
0,0 E32M47_111A2
9,8 E35M47_089A1
19,6 E32M47_203A2
24,4 E37M59_247A1
31,9 E32M47_452A2
33,6 E42M53_151A2
34,3 E42M53_146A2
37,4 E34M49_186A1
37,8 E33M49_185A1 E32M49_188A1
45,3 E34M49_412A1
50,4 E33M49_107A1
55,1 E33M49_287A1
60,8 E37M57_075A1
61,1 E42M53_170A2
61,3 E32M47_113A1
61,5 E45M49_094A1
61,6 E38M55_075A2
62,2 E35M57_242A2
63,8 E35M47_197A1 E42M55_051A1
67,8 E44M61_411A1
73,4 E34M47_442A1
78,6 E32M47_115A2 E38M53_166A2
78,7 E32M47_114A2
78,8 E38M53_158A2
78,9 E38M53_165A2 E38M53_170A2
E38M53_171A2 E44M61_412A2
79,9 E33M59_163A2
83,1 E40M55_082A2
83,4 E40M55_081A2
86,6 E44M61_196A2
89,5 E40M55_308A1
108,2 E38M61_466A2
114,8 E42M57_064A1


A3
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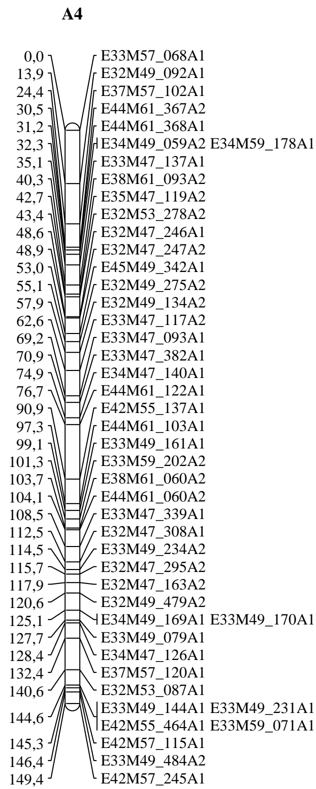
13,9 E35M47_169A1
18,0 E37M59_191A1
21,8 E32M49_202A2
31,9 E34M49_268A1
48,6 E32M49_059A2
50,4 E32M47_101A1
53,3 E34M59_135A1
59,3 E42M57_272A1
60,2 E42M53_204A1
60,6 E35M47_095A1 E35M53_124A1
E42M53_213A1 E37M57_074A1
E40M59_229A1 E40M59_230A1
E40M61_305A2 E44M61_135A1
61,0 E44M61_259A1
63,3 E42M57_099A1
64,1 E36M55_056A1
67,2 E35M57_208A2 E42M57_211A2
E33M57_211A2 E37M57_199A2
E37M57_211A2
68,6 E42M55_252A2
70,8 E38M55_184A2
71,3 E34M47_080A2 E34M47_435A2
E32M47_243A2 E36M55_187A2
E40M59_059A2 E44M61_091A2
86,4 E34M49_157A2
91,5 E35M47_150A1
101,3 E32M49_310A1
102,5 E37M57_106A1
147,8 E36M55_391A1


A4
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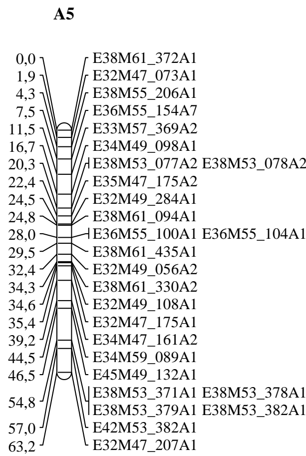
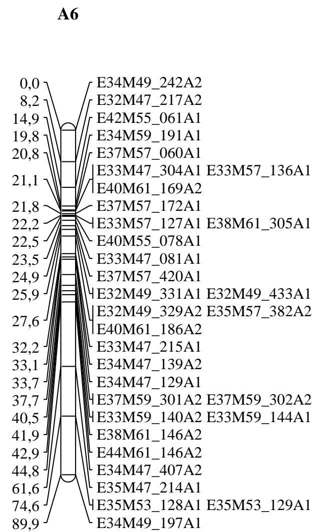
13,9 E32M49_092A1
24,4 E37M57_102A1
30,5 E44M61_367A2
31,2 E44M61_368A1
32,3 E34M49_059A2 E34M59_178A1
35,1 E33M47_137A1
40,3 E38M61_093A2
42,7 E35M47_119A2
43,4 E32M53_278A2
48,6 E32M47_246A1
48,9 E32M47_247A2
53,0 E45M49_342A1
55,1 E32M49_275A2
57,9 E32M49_134A2
62,6 E33M47_117A2
69,2 E33M47_093A1
70,9 E33M47_382A1
74,9 E34M47_140A1
76,7 E44M61_122A1
90,9 E42M55_137A1
97,3 E44M61_103A1
99,1 E33M49_161A1
101,3 E33M59_202A2
103,7 E38M61_060A2
104,1 E44M61_060A2
108,5 E33M47_339A1
112,5 E32M47_308A1
114,5 E33M49_234A2
115,7 E32M47_295A2
117,9 E32M47_163A2
120,6 E32M49_479A2
125,1 E34M49_169A1 E33M49_170A1
127,7 E33M49_079A1
128,4 E34M47_126A1
132,4 E37M57_120A1
140,6 E32M53_087A1
144,6 E33M49_144A1 E33M49_231A1
E42M55_464A1 E33M59_071A1
145,3 E42M57_115A1
146,4 E33M49_484A2
149,4 E42M57_245A1


A5
0,0 E38M61_372A1
1,9 E32M47_073A1
4,3 E38M55_206A1
7,5 E36M55_154A7
11,5 E33M57_369A2
16,7 E34M49_098A1
20,3 E38M53_077A2 E38M53_078A2
22,4 E35M47_175A2
24,5 E32M49_284A1
24,8 E38M61_094A1
28,0 E36M55_100A1 E36M55_104A1
29,5 E38M61_435A1
32,4 E32M49_056A2
34,3 E38M61_330A2
34,6 E32M49_108A1
35,4 E32M47_175A1
39,2 E34M47_161A2
44,5 E34M59_089A1
46,5 E45M49_132A1
54,8 E38M53_371A1 E38M53_378A1
E38M53_379A1 E38M53_382A1
57,0 E42M53_382A1
63,2 E32M47_207A1


A6
0,0 E34M49_242A2
8,2 E32M47_217A2
14,9 E42M55_061A1
19,8 E34M59_191A1
20,8 E37M57_060A1
21,1 E33M47_304A1 E33M57_136A1
E40M61_169A2
21,8 E37M57_172A1
22,2 E33M57_127A1 E38M61_305A1
22,5 E40M55_078A1
23,5 E33M47_081A1
24,9 E37M57_420A1
25,9 E32M49_331A1 E32M49_433A1
27,6 E32M49_329A2 E35M57_382A2
E40M61_186A2
32,2 E33M47_215A1
33,1 E34M47_139A2
33,7 E34M47_129A1
37,7 E37M59_301A2 E37M59_302A2
40,5 E33M59_140A2 E33M59_144A1
41,9 E38M61_146A2
42,9 E44M61_146A2
44,8 E34M47_407A2
61,6 E35M47_214A1
74,6 E35M53_128A1 E35M53_129A1
89,9 E34M49_197A1

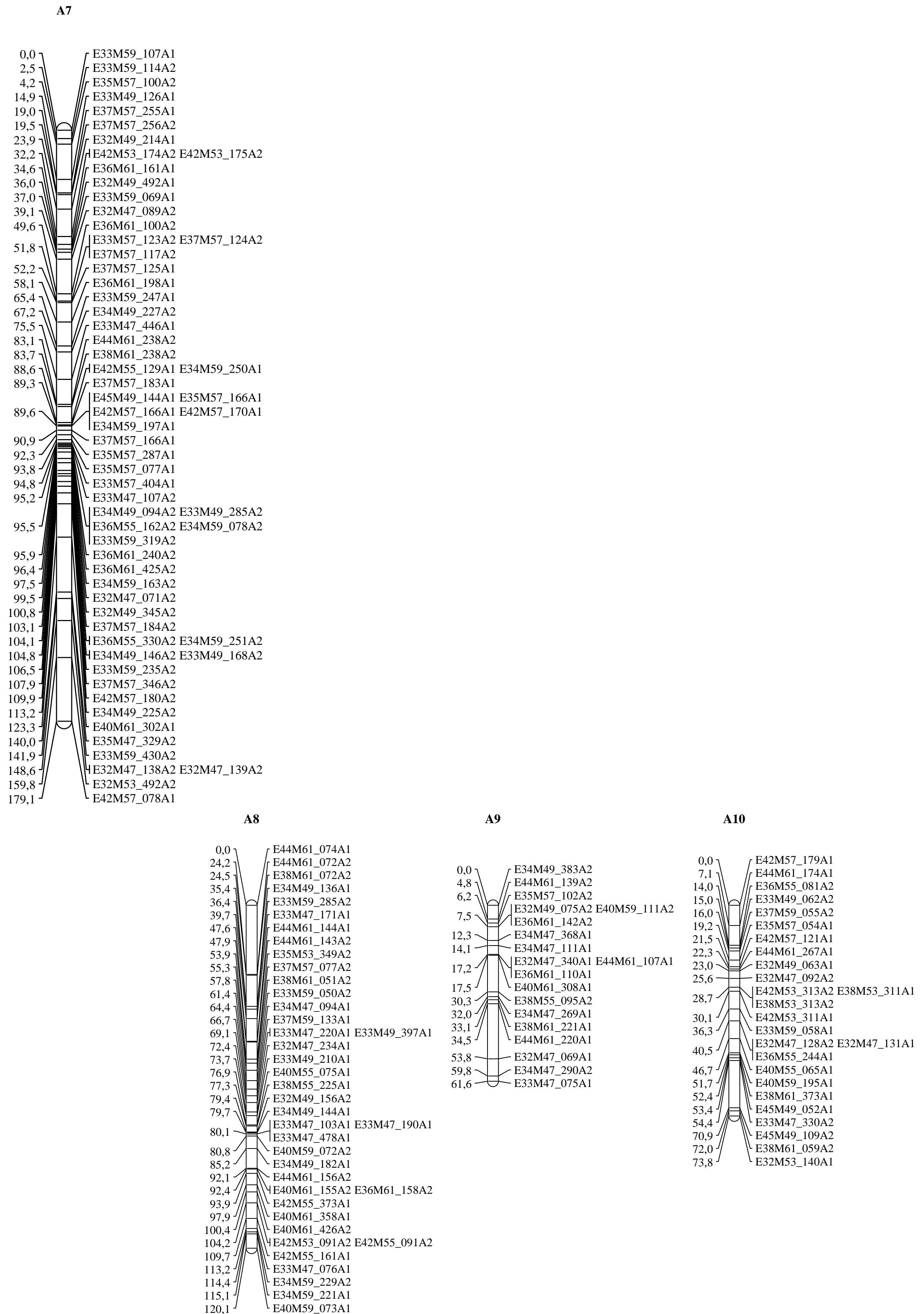
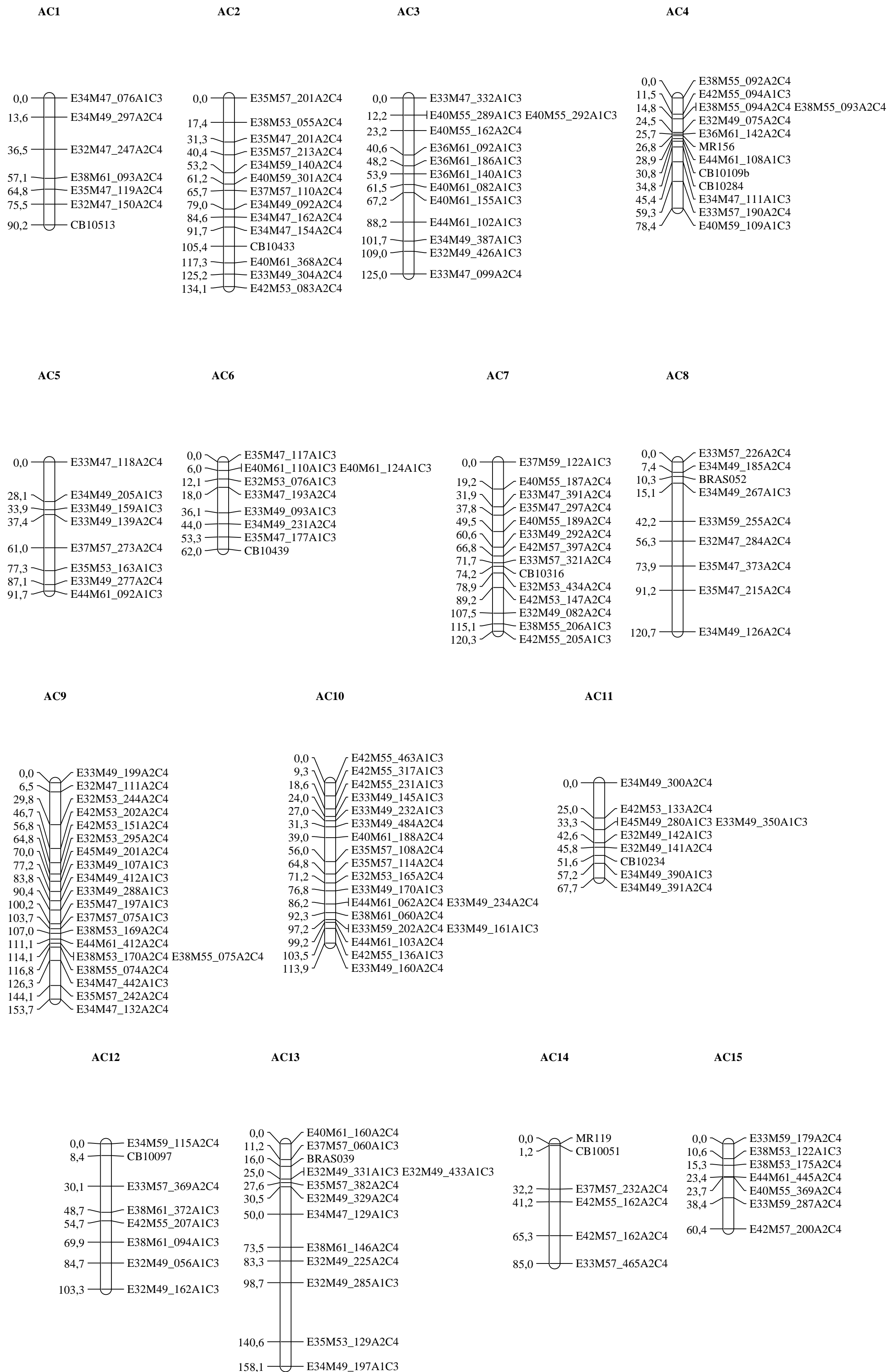


Figure 9: Genetic linkage map of *Brassica rapa* (cross A_1A_2): Marker loci are presented in absolute positions from the beginning of the linkage groups in cM.


AC1
0,0 E34M47_076A1C3
13,6 E34M49_297A2C4
36,5 E32M47_247A2C4
57,1 E38M61_093A2C4
64,8 E35M47_119A2C4
75,5 E32M47_150A2C4
90,2 CB10513
AC2
0,0 E35M57_201A2C4
17,4 E38M53_055A2C4
31,3 E35M47_201A2C4
40,4 E35M57_213A2C4
53,2 E34M59_140A2C4
61,2 E40M59_301A2C4
65,7 E37M57_110A2C4
79,0 E34M49_092A2C4
84,6 E34M47_162A2C4
91,7 E34M47_154A2C4
105,4 CB10433
117,3 E40M61_368A2C4
125,2 E33M49_304A2C4
134,1 E42M53_083A2C4
AC3
0,0 E33M47_332A1C3
12,2 E40M55_289A1C3 E40M55_292A1C3
23,2 E40M55_162A2C4
40,6 E36M61_092A1C3
48,2 E36M61_186A1C3
53,9 E36M61_140A1C3
61,5 E40M61_082A1C3
67,2 E40M61_155A1C3
88,2 E44M61_102A1C3
101,7 E34M49_387A1C3
109,0 E32M49_426A1C3
125,0 E33M47_099A2C4
AC4
0,0 E38M55_092A2C4
11,5 E42M55_094A1C3
14,8 E38M55_094A2C4 E38M55_093A2C4
24,5 E32M49_075A2C4
25,7 E36M61_142A2C4
26,8 MR156
28,9 E44M61_108A1C3
30,8 CB10109b
34,8 CB10284
45,4 E34M47_111A1C3
59,3 E33M57_190A2C4
78,4 E40M59_109A1C3
AC5
0,0 E33M47_118A2C4
28,1 E34M49_205A1C3
33,9 E33M49_159A1C3
37,4 E33M49_139A2C4
61,0 E37M57_273A2C4
77,3 E35M53_163A1C3
87,1 E33M49_277A2C4
91,7 E44M61_092A1C3
AC6
0,0 E35M47_117A1C3
6,0 E40M61_110A1C3 E40M61_124A1C3
12,1 E32M53_076A1C3
18,0 E33M47_193A2C4
36,1 E33M49_093A1C3
44,0 E34M49_231A2C4
53,3 E35M47_177A1C3
62,0 CB10439
AC7
0,0 E37M59_122A1C3
19,2 E40M55_187A2C4
31,9 E33M47_391A2C4
37,8 E35M47_297A2C4
49,5 E40M55_189A2C4
60,6 E33M49_292A2C4
66,8 E42M57_397A2C4
71,7 E33M57_321A2C4
74,2 CB10316
78,9 E32M53_434A2C4
89,2 E42M53_147A2C4
107,5 E32M49_082A2C4
115,1 E38M55_206A1C3
120,3 E42M55_205A1C3
AC8
0,0 E33M57_226A2C4
7,4 E34M49_185A2C4
10,3 BRAS052
15,1 E34M49_267A1C3
42,2 E33M59_255A2C4
56,3 E32M47_284A2C4
73,9 E35M47_373A2C4
91,2 E35M47_215A2C4
120,7 E34M49_126A2C4
AC9
0,0 E33M49_199A2C4
6,5 E32M47_111A2C4
29,8 E32M53_244A2C4
46,7 E42M53_202A2C4
56,8 E42M53_151A2C4
64,8 E32M53_295A2C4
70,0 E45M49_201A2C4
77,2 E33M49_107A1C3
83,8 E34M49_412A1C3
90,4 E33M49_288A1C3
100,2 E35M47_197A1C3
103,7 E37M57_075A1C3
107,0 E38M53_169A2C4
111,1 E44M61_412A2C4
114,1 E38M53_170A2C4 E38M55_075A2C4
116,8 E38M55_074A2C4
126,3 E34M47_442A1C3
144,1 E35M57_242A2C4
153,7 E34M47_132A2C4
AC10
0,0 E42M55_463A1C3
9,3 E42M55_317A1C3
18,6 E42M55_231A1C3
24,0 E33M49_145A1C3
27,0 E33M49_232A1C3
31,3 E33M49_484A2C4
39,0 E40M61_188A2C4
56,0 E35M57_108A2C4
64,8 E35M57_114A2C4
71,2 E32M53_165A2C4
76,8 E33M49_170A1C3
86,2 E44M61_062A2C4 E33M49_234A2C4
92,3 E38M61_060A2C4
97,2 E33M59_202A2C4 E33M49_161A1C3
99,2 E44M61_103A2C4
103,5 E42M55_136A1C3
113,9 E33M49_160A2C4
AC11
0,0 E34M49_300A2C4
25,0 E42M53_133A2C4
33,3 E45M49_280A1C3 E33M49_350A1C3
42,6 E32M49_142A1C3
45,8 E32M49_141A2C4
51,6 CB10234
57,2 E34M49_390A1C3
67,7 E34M49_391A2C4
AC12
0,0 E34M59_115A2C4
8,4 CB10097
30,1 E33M57_369A2C4
48,7 E38M61_372A1C3
54,7 E42M55_207A1C3
69,9 E38M61_094A1C3
84,7 E32M49_056A1C3
103,3 E32M49_162A1C3
AC13
0,0 E40M61_160A2C4
11,2 E37M57_060A1C3
16,0 BRAS039
25,0 E32M49_331A1C3 E32M49_433A1C3
27,6 E35M57_382A2C4
30,5 E32M49_329A2C4
50,0 E34M47_129A1C3
73,5 E38M61_146A2C4
83,3 E32M49_225A2C4
98,7 E32M49_285A1C3
140,6 E35M53_129A2C4
158,1 E34M49_197A1C3
AC14
0,0 MR119
1,2 CB10051
32,2 E37M57_232A2C4
41,2 E42M55_162A2C4
65,3 E42M57_162A2C4
85,0 E33M57_465A2C4
AC15
0,0 E33M59_179A2C4
10,6 E38M53_122A1C3
15,3 E38M53_175A2C4
23,4 E44M61_445A2C4
23,7 E40M55_369A2C4
38,4 E33M59_287A2C4
60,4 E42M57_200A2C4

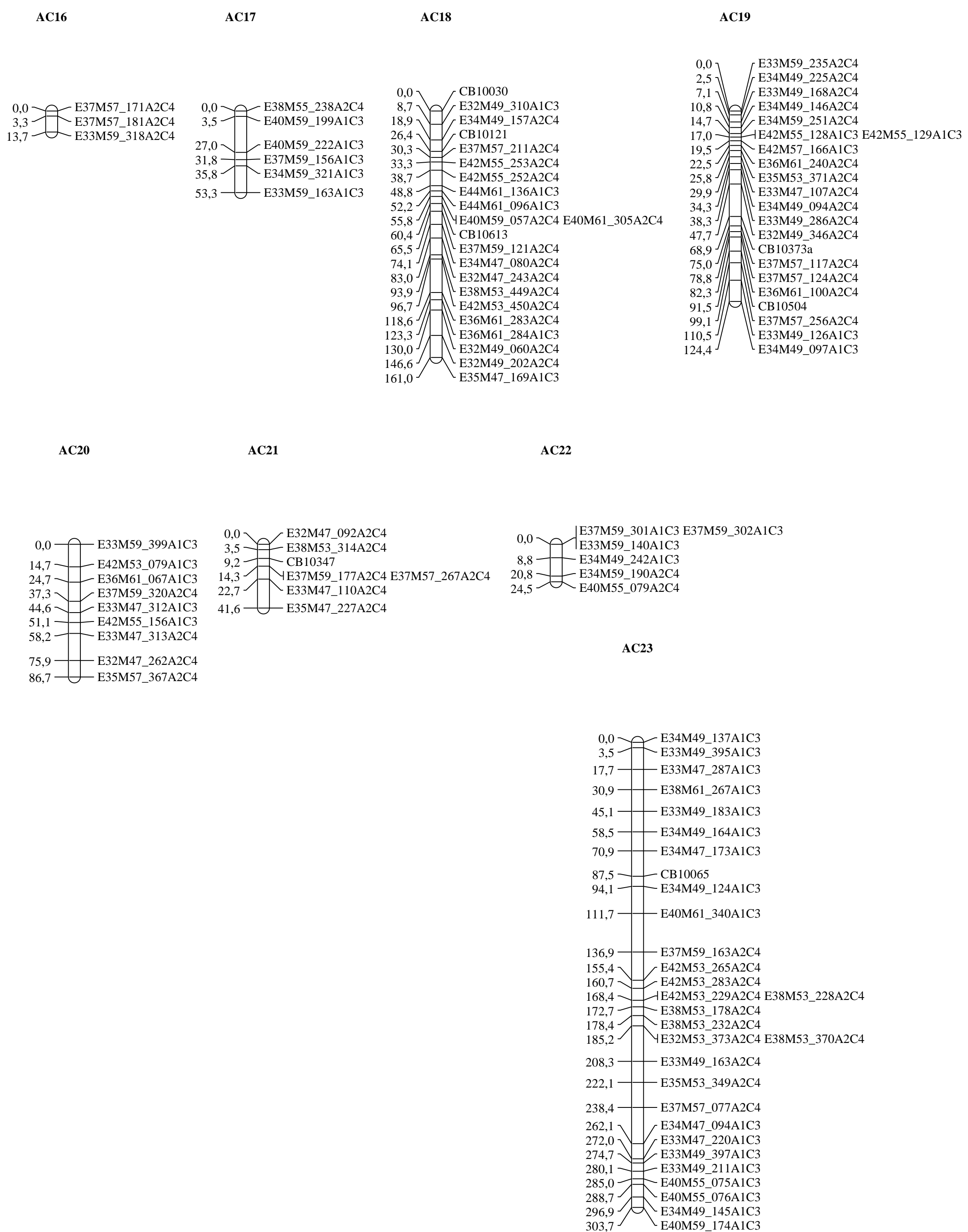


Figure 10: Genetic linkage map of *Brassica napus* ($A_1A_2C_3C_4$): Marker loci are presented in absolute positions from the beginning of the linkage groups in cM.)

4.3.3 QTL-Analysis and Comparison

The results of the main effect QTL analyses for early fresh matter and dry matter biomass were summarised and the corresponding values compared for diploid and allopolyploid situations in Table 7.

A negative signed effect means that the marker derived from parent two and a positive signed one from parent one. In the *Brassica rapa* population 18 QTL with a LOD score more than 3.0 were detected whereas in the *Brassica oleracea* population 30 significant QTL could be counted. A total of 29 QTL at a LOD score over 3.0 could be detected in the allopolyploid population. Besides for the traits FM2-FM1 and DM1 for the *Brassica rapa* population QTL could be localized for each trait in the three RIL populations.

For the trait FM1 the significant QTL explained 23.8% of the phenotypic variance in *B. rapa*, 55.26% in *B. oleracea* and 30.72% in *B. napus*. For FM2 the total explained variance is 33.31%, 21.77% and 15,08%, and for FM2-FM1 0%, 73.48% and 29.17%. No phenotypic variance could be explained by significant QTL for DM1 in *B. rapa*, 84.69% in *B. oleracea* and 32.35% in the allopolyploid. DM2 is explained by 32.47%, 67.49% and 20.17%. Variance of growth rate DM2-DM1 was described by 33.68%, 68.94% and 11.81%.

Table 7: QTL and their main effects in the diploid populations (A_1A_2, C_3C_4) compared with the ones occurred in the allopolyploid ($A_1A_2C_3C_4$)

		QTL in the diploids						QTL in $A_1A_2C_3C_4$					
Genotype	trait	LG		Pos [cM]	Vp [%]	A	LOD	LG		Pos [cM]	Vp [%]	A	LOD
A_1A_2	FM1	4		116.9	3.22	48.7	3.18	10		64.8	3.82	-81.9	7.19
		3	=N06	95.6	1.70	-35.4	1.33	18	=N16	38.1	10.65	-136.8	15.80
								4		71.4	8.22	-120.2	8.02
		2		17.8	5.72	64.9	4.16	-					
		2		78.3	5.27	-62.3	4.00	-					
		7		2.0	7.90	-76.3	5.46	19					
	FM2	3	=N06	8.0	4.14	113.3	2.32	18	=N06	49.1	2.27	-116.0	5.30
		1	=N07	38.0	9.05	-167.7	6.72	6	=N07				
		2		78.3	9.53	-172.0	6.24	9					
		7		21.5	9.59	-172.7	5.68	19		43.7	0.95	75.2	2.30
		9						4	=N10	61.4	2.57	-123.5	3.74
	FM2-FM1	1						6	=N07	4.0	7.15	133.1	9.74
		1						6	=N07	49.3	4.56	-106.3	5.99
		3						18	=N06	49.1	3.86	-97.8	5.19
	DM1	6						13	=N08	76.2	8.30	-8.5	16.24
		3						18	=N06	41.5	5.64	-7.0	11.95
	DM2	7		23.9	6.61	-10.3	7.64	19		10.3	1.77	7.5	3.31
		7		142.5	8.52	11.7	8.06	19					
		1	=N07	40.0	2.71	-6.6	3.16	6					
		2		78.3	1.80	-17.0	14.65	9					
		4		119.8	3.89	7.9	4.34	10		24.0	2.84	-9.5	5.05
		8		63.8	2.96	6.9	3.05	-					
		8		102.2	5.98	-9.8	5.35	-					
		3						18	=N06	50.6	7.00	-14.8	7.19

		QTL in the diploids						QTL in $A_1A_2C_3C_4$					
Genotype	trait	LG		Pos [cM]	Vp [%]	A	LOD	LG		Pos [cM]	Vp [%]	A	LOD
	DM2-DM1	1	=N07	37.0	5.34	-6.5	3.68	17	=N07	23.5	4.50	-7.9	8.97
		7		146.5	7.30	7.6	5.71	19		43.7	0.221	5.5	6.28
		2		78.3	15.86	-11.2	10.18	9					
		8		57.2	5.18	6.4	3.53	-					
C_3C_4	FM1	2	=N12	382.7	6.20	-40.0	2.26	7	=N12	107.6	8.03	118.8	12.32
		6		58.1	6.09	-39.6	3.13	5					
		7	=N17	386.2	8.07	45.6	4.06	2					
		4		376.2	15.22	62.7	3.28	-					
		9		42.6	13.33	58.7	5.84	-					
		14		83.0	6.35	40.5	3.27	-					
	FM2	2	=N12	75.3	2.78	-45.8	1.76	7	=N12	107.6	3.33	140.5	7.46
		3	=N15	173.5	2.46	-44.7	2.52	8	=N15	6.0	3.17	-137.1	6.97
		6		4.8	5.77	68.6	4.84	5		20.0	2.77	-128.2	4.32
		14		83.0	10.82	93.9	9.51	-					
	FM2-FM1	6		4.8	8.11	54.4	6.17	5		2.0	2.17	-73.4	3.65
		6						5		85.3	1.97	-69.9	3.45
		2	=N12	73.3	6.01	-46.8	3.99	7					
		3	=N15	38.9	4.33	-39.8	3.45	8	=N15	7.5	0.39	-31.1	0.74
		7		138.6	5.53	44.9	4.10	20					
		9		77.2	37.75	117.4	12.06	-					
		11		18.1	4.43	-40.2	3.16	-					
		13		89.5	7.21	51.3	3.35	-					
		1						2	=N17	46.4	6.27	-124.6	5.06
		1						2	=N17	129.9	2.80	83.3	4.90

		QTL in the diploids						QTL in $A_1A_2C_3C_4$					
Genotype	trait	LG		Pos [cM]	Vp [%]	A	LOD	LG		Pos [cM]	Vp [%]	A	LOD
	DM1	2	=N12	73.3	27.43	-5.8	16.59	7	=N12	106.7	8.12	08.4	17.39
		3	=N15	167.6	15.51	-4.4	10.90	8	=N15	7.5	10.29	-9.4	15.85
		7		384.2	5.39	2.6	5.66	20					
		4		376.2	23.36	5.4	9.47	-					
		9		34.5	3.93	2.2	4.42	-					
		12		10.8	3.00	-1.9	3.60	-					
		14		72.9	6.07	2.7	8.15	-					
	DM2	2	=N12	75.3	37.31	-14.3	11.22	7	=N12	107.2	4.27	11.6	7.08
		6		2.8	9.02	7.0	6.26	5					
		9		22.5	13.84	8.7	4.03	-					
		14		72.9	7.32	6.3	5.35	-					
		3						8	=N15	6.0	4.29	-11.6	6.78
	DM2-DM1	2	=N12	71.3	25.15	-8.7	17.45	7					
		6		2.8	5.85	4.2	7.45	5					
		10		4.0	10.16	-5.6	7.03	-					
		10		278.9	22.16	8.2	8.36	-					
		11		22.1	5.62	-4.1	6.86	-					
		1						2	=N17	127.9	5.02	8.3	11.06
		8						23		161.2	2.07	-5.3	4.06

Pos: Positions are measured in cM from the start of the linkage group; Vp: Explained phenotypic variance in %; A: Effect on the early biomass in mg per plant

4.3.4 LOD graphs

The LOD score was calculated over the whole linkage groups for all three populations and the six investigated traits (Figure 11-13). In the *B. oleracea* population for most main effect QTL detected with the program QTLMAPPER a peak in the LOD graphs derived from the program R/QTL occurred (Figure 11), besides on linkage group 11 for the trait FM2-FM1 and linkage group 6 and 14 for DM2. On linkage group 1 which could be located on N07 are peaks for FM2-FM1. For the linkage group 17 of the alloploid (Figure 13) that corresponds with N07 a peak for the trait FM2-FM1 occurred as well.

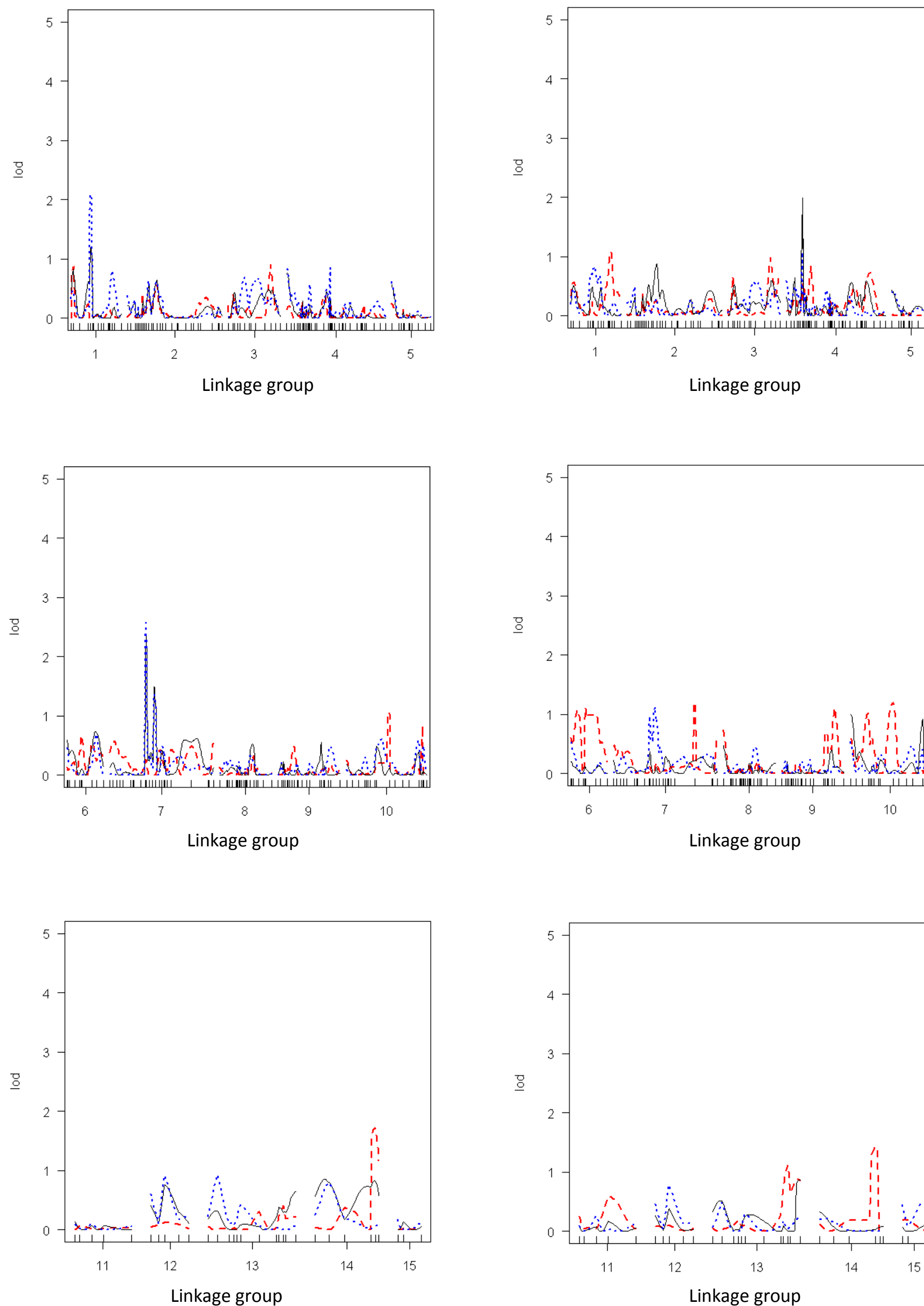


Figure 11: LOD graphs for C_3C_4, left side for the traits FM (red line FM1, black line FM2, blue line FM2-FM1) and right side for DM (red line DM1, black line DM2, blue line DM2-DM1), generated with Rqtl

The peaks of the LOD score for *B. rapa* are mainly consistent with the QTL found by MAPMAKER (Figure 12). But in the Rqtl graphs peaks appear for the traits FM2-FM1 on linkage groups were QTL for FM1 were detected - but with a lower LOD score. The graphs show peaks for DM1 in the same regions as QTL

for DM2 and especially for DM2-DM1 has been detected by MAPMAKER (linkage groups 2 and 7).

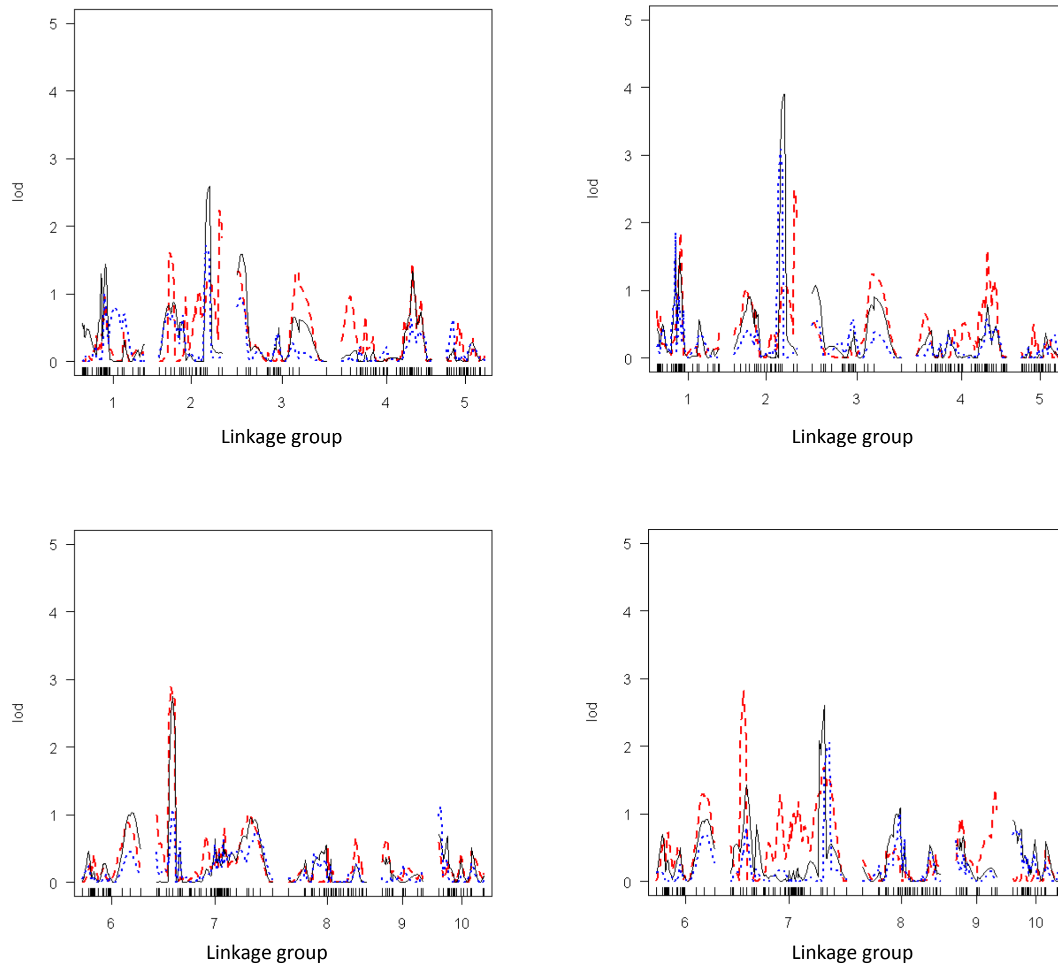


Figure 12: LOD graphs for A_1A_2, for the traits FM (red line FM1, black line FM2, blue line FM2-FM1) and right side for DM (red line DM1, black line DM2, blue line DM2-DM1), generated with Rqtl

In the allopolyploid situation for every main effect QTL detected via MAPMAKER a peak within the expected region exists in the Rqtl graphs (Figure 14). Additionally at linkage group 5 a peak occurred. This is consistent with the QTL detected in the homoeologous region in *B. oleracea*.

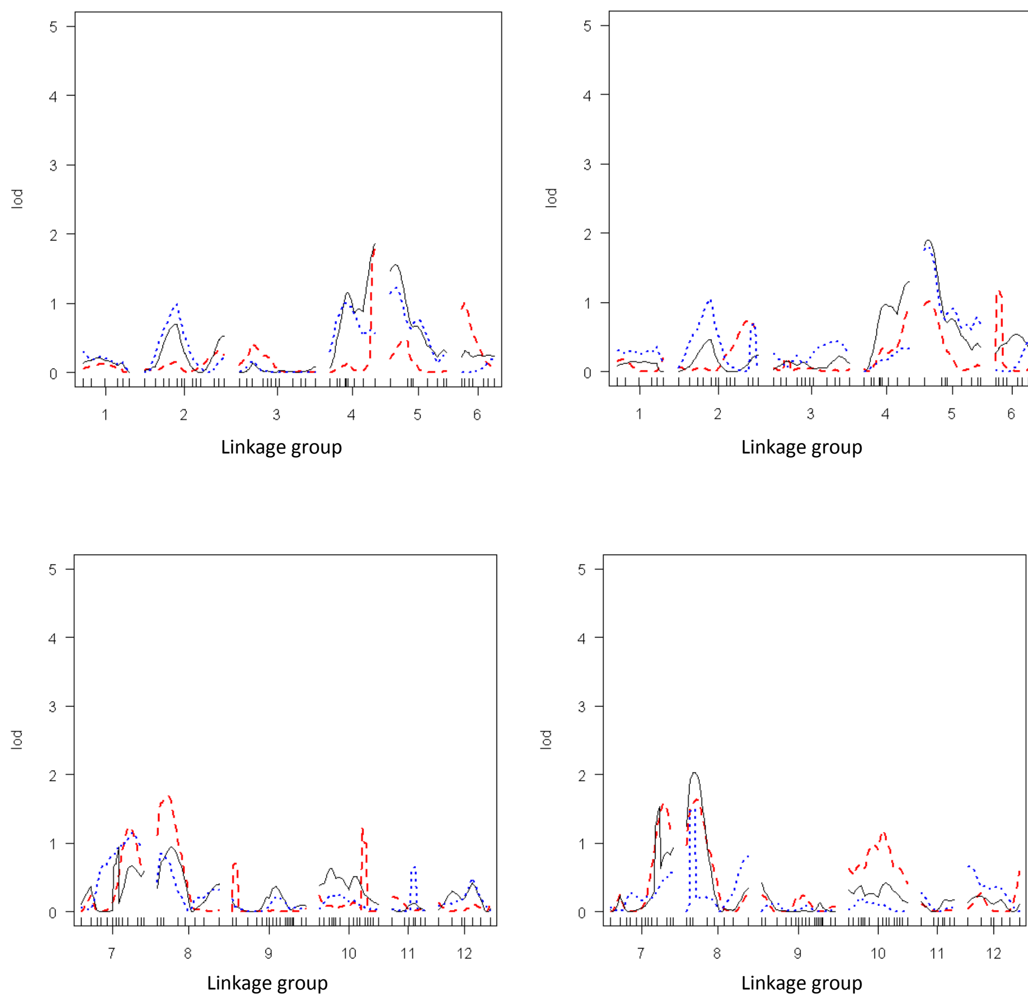


Figure 13: LOD graphs for $A_1A_2C_3C_4$, for the traits FM (red line FM1, black line FM2, blue line FM2-FM1) and right side for DM (red line DM1, black line DM2, blue line DM2-DM1), generated with Rqtl, linkage groups 1-12;

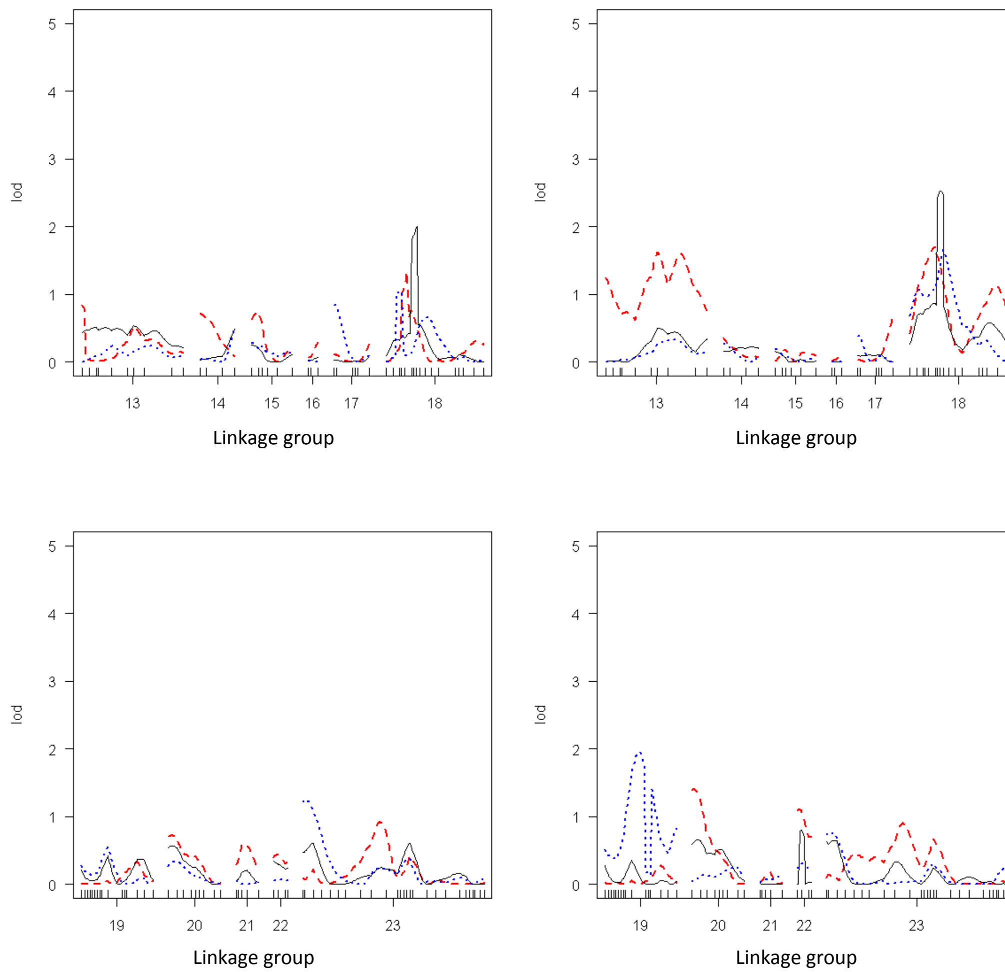


Figure 14: LOD graphs for $A_1A_2C_3C_4$, for the traits FM (red line FM1, black line FM2, blue line FM2-FM1) and right side for DM (red line DM1, black line DM2, blue line DM2-DM1), generated with Rqtl, linkage groups 13-23;

4.3.5 Analyses of Epistatic Interactions

In the QTL mapping with the allopolyploid RIL population 64 pair of loci showing digenic epistatic interactions were detected (Table 8). For nine epistatic loci pairs one or two additive effects were significant. The epistatic effects for 29 pair of loci showed negative effects, meaning that this recombination of the parental lines decreased early plant biomass. For the trait FM1 three pairs of loci correspond with loci detected for the trait FM2. For FM2-FM1 only one loci pair was the same as in FM2. For DM2 and DM1 no loci pairs corresponded, but one of DM1 accorded with FM1 and FM2 and for DM2 several loci pairs corresponded with other traits or with loci pairs directly neighboured. Also the epistatic loci pairs found for DM2-DM1 accorded with pairs found in FM2-FM1 and DM2. Five pairs of loci included one locus that showed a main effect QTL. One epistatic interaction has been detected between the homeologous linkage groups N07 and N17.

Table 8: Putative epistatic QTL detected in the allopolyploid for the traits fresh matter (FM) and dry matter (DM) for two harvest times and the growth rates (FM2-FM1, DM2-DM1)

Trait	LG		Pos	LG		Pos	LOD	Ai		Aj		Aaij	
FM1	1	-	2,0	2	=N17	31,3	3,41	-4,45		-1,84		-99,49	***
	2	=N17	77,7	5	-	91,1	4,02	34,18		34,51		84,44	***
	2	=N17	121,3	6	=N07	0,0	3,71	15,15		16,64		-87,32	***
	8	=N15	44,1	21	=N04	31,8	6,84	-42,20		13,02		-160,65	***
	8	=N15	117,4	15	-	25,4	4,42	-3,92		-11,78		125,73	***
	9	-	89,8	20	-	14,7	3,29	-2,93		-9,97		-89,16	***
	11	=N16	43,7	21	=N04	0,0	3,1	-22,96		-35,24		-68,43	***
	13	=N08	93,4	23	-	217,0	4,34	41,68		-19,12		123,09	***
FM2	3	-	50,2	15	-	14,2	3,49	3,47		60,05		216,89	***
	6	=N07	21,3	23	-	298,1	3,63	11,41		17,14		179,91	***
	7	=N12	66,2	23	-	234,8	3,04	34,73		-3,58		-134,79	***
	7	=N12	117,3	13	=N08	11,2	4,02	43,99		2,60		161,20	***
	8	=N15	42,1	21	=N04	25,8	6,79	12,10		18,19		-273,87	***
	8	=N15	77,8	20	-	14,7	5,38	12,03		65,22		-228,92	***
	8	=N15	117,4	15	-	23,4	3,64	42,69		24,74		138,98	***
	9	-	70,0	13	=N08	21,4	2,86	-10,38		31,12		136,90	***
	10	-	0,0	23	-	58,4	3,49	37,85		1,57		171,41	***
	13	=N08	93,4	23	-	217,0	4,85	14,49		-19,43		204,10	***
	15	-	25,7	16	-	0,0	4,58	15,03		-23,34		195,58	***
FM2-FM1	2	=N17	0,0*	5	-	0,0	3,65	43,96		-7,54		-87,73	***
	2	=N17	55,3	11	=N16	41,7	4,58	-21,36		-17,17		-111,86	***
	5	-	4,0*	18	=N06	67,3	4,25	-12,15		30,89		-119,19	***
	5	-	59,4	10	-	103,5	4,67	-12,98		-20,06		103,26	***
	6	-	51,3*	19	=N09	92,9	4,03	-35,78		-48,58	**	94,07	***
	7	-	66,8	23	-	193,9	4,63	-87,90	*	60,51		-164,22	***
	7	-	70,8	23	-	275,0	5,02	-29,37		36,26		-109,32	***
	8	=N15	117,4	15	-	14,6	5,28	25,82		-33,20		106,90	***

Trait	LG		Pos	LG		Pos	LOD	Ai		Aj		Aaij	
FM2-FM1	9	-	22,5	10	-	84,9	3,69	40,72		-1,06		102,23	***
	11	=N16	55,4	12	=N01	50,8	4,82	1,88		-6,63		112,20	***
	12	=N01	80,8	14	=N05	4,0	4,88	-33,99		14,63		-113,74	***
	15	-	23,4	23	-	2,0	4,12	-4,69		13,95		114,13	***
	15	-	25,7	16	-	0,0	3	8,81		-20,68		93,51	***
	17	=N07	0,0	18	=N06	49,1	7,38	-42,12		-114,88	***	75,71	**
	18	=N06	54,6	18	=N06	79,1	6,36	-233,83	***	96,31	**	171,95	**
DM1	1	-	31,5	18	=N06	0,0	4,26	0,17		0,88		-7,15	***
	4	=N10	13,5	4	=N10	29,2	4	-0,40		-0,83		8,54	***
	6	=N07	11,3	16	-	1,0	3,43	-1,78		0,83		-5,62	***
	8	=N15	115,4	15	-	10,6	4,55	-1,32		0,69		7,52	***
	10	-	66,2	23	-	30,9	3,7	0,86		-1,48		6,87	***
	13	=N08	141,3	21	=N04	13,7	3,16	-0,13		-2,37		6,90	***
	18	=N06	49,1*	21	=N04	13,7	4,42	-0,54		-1,51		-7,04	***
	20	-	0,0	23	-	0,0	2,88	2,49		-0,21		5,41	***
DM2	3	-	101,7	23	-	157,9	4,68	2,85		3,02		16,17	***
	4	=N10	61,4	7	=N12	86,9	5,01	-0,31		7,55	*	10,09	**
	6	=N07	35,4	23	-	275,3	3,11	0,30		3,83		9,32	***
	7	=N12	66,8	23	-	205,0	6,27	3,95		1,64		-11,27	***
	8	=N15	42,1	21	=N04	33,8	5,46	-3,90		2,16		-16,07	***
	8	=N15	77,8	20	-	16,7	6,93	3,68		4,56		-17,56	***
	10	-	0,0	18	=N06	54,6	6,06	-2,50		-13,65	***	-13,27	***
	10	-	2,0	23	-	57,0	5,68	-1,54		4,77		14,47	***
	13	=N08	70,2	16	-	8,0	8,28	-2,01		0,89		18,11	***
	13	=N08	79,0	23	-	206,0	3,35	0,70		-1,28		13,11	**
	18	=N06	70,2	21	=N04	15,7	4,79	-6,32		-1,55		-11,99	***

Trait	LG		Pos	LG		Pos	LOD	Ai	Aj		Aaij	
DM2-DM1	1	-	66,7	10	-	0,0	4,71	1,06	-0,22		9,35	***
	2	=N17	0,0	5	-	2,0	5,27	2,62	-0,03		-9,79	***
	3	-	12,2	15	-	23,7	4,26	2,95	3,44		9,48	***
	5	-	87,1	13	=N08	76,2	3,11	-2,50	0,34		-6,39	***
	7	=N12	66,8	23	-	269,0	3,49	0,94	4,88	*	-5,43	**
	7	=N12	109,6	10	-	80,9	5,29	1,14	0,82		10,04	***
	7	=N12	119,3	22	-	6,0	4,42	1,63	0,15		-8,61	***
	8	=N15	66,2	20	-	14,7	4,69	3,02	6,56	***	-9,80	***
	11	=N16	62,4	12	=N01	54,8	5,51	1,35	-4,93	*	8,89	***
	15	-	27,7	16	-	0,0	5,36	5,02	-1,00		10,88	***
	16	-	0,0	22	-	9,1	7,73	-1,09	-0,92		-11,96	***

LGi and LGj are the corresponding linkage groups
A_i, A_j: main effects at loci I and j; AAij epistatic interaction effect between loci i and j in mg
*significant at P = 0.05; ** significant at P = 0.005; *** significant at P = 0.001
Positions are measured from the beginning of the linkage group in cM

4.4 Discussion

4.4.1 Material and biomass trials

For fixed heterosis - instead of a comparison of F1-plants with their parental lines used for classical heterosis trials - the resynthesized lines were compared with two populations of RIL/homozygous genotypes of the parental lines. A direct comparison between the parental lines and the hybrid as normally done for classical heterosis was not possible here. Else wise for every parental combination in the RIL populations a resynthesized rapeseed should have been created.

In this study instead of autotetraploid lines the diploid A_1A_2 and C_3C_4 lines were used for biomass trials and for a comparison with the resynthesized $A_1A_2C_3C_4$ lines. This results from the fact that the autotetraploid lines were not as fertile and vigorous as the diploid ones. Abel and Becker (2007) observed that the tetraploid lines yielded 2–64% less than the corresponding diploid lines. Difficulties were expected for the development of RILs and due to meiotic complications also in the marker analysis and the interpretation of results.

Diploid and allopolyploid forms showed a similar dry matter content what corresponds with the observations of Abel and Becker (2007) that the ploidy level had no significant effect on the dry matter content.

The trait early plant biomass was chosen due to the fact that resynthesized lines often do not show a comparable amount of yield. In our study some of the lines showed abnormal flower forms or did not produce the expected amount of pollen. A high correlation between early plant biomass and yield was described by Radoev (2007). Early biomass trials are more cost effective and due to regulated conditions in the greenhouse it is possible to have more than one generation per year instead of one generation in field trials. Besides this the selfing of some plants produced so less seeds, that the greenhouse was more secure to get viable plants and evaluable results than a field trial with environmental interactions.

For the trait early plant biomass the seed size plays an essential role (Meyer et al. 2004). In this trial we did not determine the seed size of the RIL but a wide variation could be observed. To minimize an impact of the seed size, comparable growing conditions, a sufficient amount of water and nutrients were available for the seedlings during the trials.

As the trials took place in the greenhouse the conditions for the four replicates were the same, although they were time-lagged. The three RIL populations showed a difference in their growth behaviour. The *B. rapa* types grew faster than the *B. oleracea* types – about 19% more biomass production at the second harvest compared to *B. oleracea.* This could be due to the different habitus of *B. rapa* and *B. oleracea.*

Dry matter and fresh matter was measured at two times to determine the growth rate. In other studies only the dry matter (Meyer et al. 2004) or the fresh matter (Liu et al. 2002) was used as a trait because of the close correlation between these two traits. In this study a close correlation from 0.72 to 0.97 could be observed but in the QTL analyses different putative main effect QTL could be detected for dry matter and fresh matter traits. The proportion of water in the plants is possibly not regulated by the same traits as the dry matter is.

For calculation of QTL within the three populations it is necessary to have differences between the RILs of a mapping population. This is important to enhance the possibility of identifying a large set of polymorphic markers that are well-distributed across the genome. Recombination events linked with differences in biomass yield are used to identify QTL. These requirements were met as the variation between genotypes within one population was highly significant. In our study the different lines of the A_1A_2 population showed a higher variability than the RIL of the C_3C_4 population. This agrees with the Jaccard's genetic distances of 0.741 for A_1 and A_2 und of 0.313 for C_3 and C_4 calculated by Abel et al. (2005). The biggest difference between the minimum and maximum values and the highest variance components over all traits could be observed in the alloploids (Table 4). The mean values of the alloploids are significantly higher than the mean values for early plant biomass of the diploids therefore this plant material can be used to identify QTL for fixed heterosis.

4.4.2 Creation of linkage maps and QTL analyses

The marker analysis was performed with AFLP and SSR. AFLPs are a cost effective and fast technique for detecting polymorphisms in DNA and for developing a framework map (Vos et al. 1995). In addition some SSR markers that are distributed equally over the genome were needed to align the results with other linkage maps. In former studies saturated mapping of microsatellite markers in *B. oleracea*, as well as in *B. rapa* and *B. napus*, could be successfully used to reveal the homoeologous relationships (Saal et al. 2001). A linkage map based on molecular markers that would allow discrimination between homologous and homeologous regions is required for a comparative analysis in *Brassica*.

The linkage map for *B. oleracea* had a size of 1509.1 cM and consisted of 15 linkage groups. These results are comparable with other maps (Cheung et al. 1997) that had a length of 1546.1 cM and consisted of 297 markers. An integrated map of two *B. oleracea* populations consisted of 547 markers, 212 deriving from AFLP, and had a total length of 893 cM (Sebastian et al. 2000).

For *B. napus* the linkage map resulted in a length of 2373.4 cM and a total number of 297 AFLP markers could be mapped. In a study on a consensus linkage map construction Lombard and Delourme (2001) estimated a probable range of the rapeseed genome length from 2.127 cM to 2.480 cM. Piquemal et al. (2005) applied a consensus mapping approach and developed a linkage map of *B. napus*, that covered 2.619 cM. This was beyond the range estimated by Lombard and Delourme (2001). A shorter linkage map of rapeseed was published by Cheung et al. (1997), covering 1.954.7 cM for 19 major linkage groups and 2.124.9 cM by including ten unassigned fragments of less than four markers.

In *B. rapa* 377 AFLP marker were used of which 93 showed a close linkage to others. So the framework map consisted of 284 informative markers. The resulting map had a length of 1081cM distributed among 10 linkage groups. A reference genetic linkage map for the *Brassica* A genome has been constructed that consists of a total of 556 markers (Choi et al. 2007). The total length of this linkage map was 1182 cM. In former studies the distances of genetic linkage maps ranged from 890 cM up to 1850 cM (Chang et al. 2008).

Map sizes are not easily comparable, since they are influenced by the degree of genome coverage by marker loci, the size and type of mapping population, the applied mapping function, the used software and the amount of recombination

events. Those are influenced by the genetic diversity of the parents and/or environmental effects on meiosis (Ferreira et al. 1994).

Main effect QTL detected in the *B. oleracea* population explained more phenotypic variation than QTL found in the other two populations. The percentage of explained variation could be higher in comparison to the *B. rapa* and *B. napus* populations as the parental lines C_3C_3 and C_4C_4 are closer related and differ in less alleles (Abel et al. 2005). It is thus not possible to decide wether the variation left unexplained is due to other QTL or the environment (Kearsey and Farquhar 1998).

Different thresholds for the determination of main and epistatic QTL were used because the aim of the project was to identify main QTL that occur in the allopolyploid as well as in the diploids despite their explained variance. For the epistatic QTL a stricter threshold was used because a threshold of P =0.005 resulted in epistatic QTL that showed no significant effects.

4.4.3 Epistatic interactions

Because homozygous lines are used, only additive effects and additive x additive epistasis can occur. With only an additive gene action (no epistasis/fixed heterosis) all QTL detected in the A and C genome should have the same effect in the allopolyploid situation. So if the genetic variance of the two homozygous RIL populations is defined as $\Sigma\, a_A^2$ and $\Sigma\, a_C^2$, the genetic variance of the allopolyploid population is expected to be $(\Sigma\, a_A^2 + \Sigma\, a_C^2)$. In case of deviation from the expected genetic variance in the allopolyploid situation this would be an evidence for epistatis.

A lot of epistatic interactions were expected as the progenitor diploid genomes (A and C) of the amphidiploid *Brassica napus* are extensively duplicated with 73% of genomic clones detecting two or more duplicate sequences within each of the diploid genomes (Parkin et al. 2003). This comprehensive duplication of loci is to be expected in a species that has evolved through a polyploid ancestor. Replicated genes are often not expressed due to gene silencing (Lukens et al. 2003), but nevertheless epistatic interactions between duplicated genes might be much more important than considered so far.

Most epistatic QTL were detected between loci that did not show a main effect QTL. This could be due to the fact that some effects of the main QTL are overestimated. Besides, there are more possibilities for epistatic interactions that could have an effect, than for main effect QTL in a genome. The number of estimated effects also influences the significance level.

One epistatic interaction has been detected between the homeologous linkage groups N07 and N17. But most epistatic QTLs are between linkage groups that could not been aligned with other maps or were between loci on the same linkage group. These interactions were expected between duplicated regions on the same chromosomes or the homologe ones due to the knowledge about the relationship of *Brassica* genomes (U 1935).

Basunanda et al. (2010) could effectively demonstrate that numerous heterosis-related QTL for different yield-related traits in *B. napus* appear to overlap at the same or similar positions in different genetic backgrounds. Particularly prevalent QTL hotspots, often with corresponding homoeologous QTL, were detected in the different populations on linkage groups N01/N11, N2/N12, N03/N13, N05/N15, N06, N07/N16 and N10/N19.

Comparing our results with that of Basunanda et al. (2010) we could also find QTL for early plant biomass in the 3 RIL populations near this “hot spots” for seed yield and heterosis on the linkage groups N06, N07 and N16.

4.4.4 Relevance for Fixed heterosis

In the literature QTL controlling flowering time in *Brassicas* have been found to map to similar regions in homoeologous chromosomes both within and between species (Lagercrantz et al. 1996; Osborn et al. 1997). In our study we compared QTL for early plant biomass between diploid and alloploid *Brassica* species.

As to our assumptions some QTL found in the diploid lines disappeared in the allopolyploid or had no significant effect. Also additional QTL could be detected in the allopolyploid. This was possibly due to the different densities of the maps and the different number of genotypes tested. Due to homeologous or epistatic interactions other regions could influence the traits in the allopolyploid situation.

Most of the observed QTL were of type I and type II loci (Figure 7) and the effect of a better performance of the allopolyploid could be due to positive epistatic effects.

Type III loci were found for the homoeologous linkage groups N06 and N16, and N07 and N17 for the *B. rapa* and the *B. oleracea* population. In the *B. napus* population only for N06 main effect QTL could be detected but not for N16. In the LOD graphics occurred a peak but the corresponding linkage group in the allopolyploid has only 67.7 cM and possibly not covering the whole chromosome. For N07 the allopolyploid showed a main effect QTL for the traits FM2-FM1 and DM2-DM1 and for N17 as well.

In linkage groups N07 and N17 an epistatic interaction could be detected in the allopolyploid. Parkin et al. (2003) described primary regions of homoeology between the linkage groups N15 and N06, N06 and N17, and N17 and N07. Also homoeologous polymorphic marker loci have been detected between linkage groups N07 and N16 by Quijada et al. (2006) and Udall et al. (2006). But it was not possible to allocate all linkage groups of the three populations to each other so that there are possibly more homoeologous interactions that were not defined as such. For further studies more SSR markers that are tested in the same amount of lines as the AFLP markers should help to reveal the homoeologous relationships.

It was not possible to attribute fixed heterosis to epistatic interaction. So for the detected putative main effect QTL that corresponded in the homoeologous regions no significant epistatic interaction was found. But comparisons with the LOD graphs show that there are several areas not significant in the QTLMAPPER analyses, that show a peak at a corresponding chromosome, so possibly more corresponding minor QTL are there. But due to the significance limit or an over- or underestimation of other QTL or main effect markers they could not been detected.

4.4.5 Recombinations in resynthesized rapeseed

The fact that there had been difficulties in later selfing generations and self incompatibility occurred besides the parental lines did not show this, lead to the assumption that instabilities in the allopolyploids may have occurred during meiosis. Also the high number of markers for which a positioning within the genetic map was not possible due to a skewed segregation could be a hint for genomic changes. Tate at al. (2006) stated that most of the cDNA-AFLP polymorphisms apparently resulted from loss of parental fragments in the polyploids and that changes at the genomic level have occurred stochastically among individuals within the independently formed populations. Parkin et al. (2003)observed that the majority of the duplicate loci within each of the diploid genomes were found in distinct linkage groups as collinear blocks of linked loci, some of which had undergone a variety of rearrangements subsequent to duplication, including inversions and translocations. Udall et al. (2005) suggest that chromosomal rearrangements caused by homeologous recombination are widespread in *B. napus*. Also in *Arabidopsis* whereas wild allopolyploids are well adapted, man-made allopolyploids are typically unstable, displaying homeotic transformation and lethality as well as chromosomal rearrangements and changes in the number and distribution of repeated DNA sequences within heterochromatin. Large increases in the length of some chromosomes has been documented in allopolyploid hybrids and could be caused by the activation of dormant retrotransposons, as shown to be the case in interspecific hybrids (Comai 2000).

Insights into this genome functions gained from the study of allopolyploidy may be applicable to hybrids of any type and may even elucidate positive interactions, such as those responsible for hybrid vigor.

5 Outlook

The Brassica family is a good model plant to investigate the different types of chromosomal interactions. So interactions between homoeologous chromosomes as well as between homologous chromosomes and epistatic interactions occur. Due to the many possibilities in ploidy level of the Brassica family and the possible crossings we were able to observe intergenomic dominance and dosage effects. It allowed us to identify at least some of the QTL for fixed heterosis which can then be analysed in detail in further investigations. Due to the use of SSR markers it was possible to align homoeologous chromosomes and compare these results with the results of QTL mapping in other groups working on *Brassica* (Quijada et al. 2006; Udall et al. 2006; Basunanda et al. 2010). The results of the QTL analysis will contribute to understanding the influence of fixed heterosis on the success of alloploids.

To get a deeper insight in mechanisms responsible for the genomic interactions in hybrids further investigations will be necessary. One possibility could be to use an illumina chip to compare extensively the parental and the resynthesized lines. Comparative epigenetical studies in the A, C and the alloploid genome could help to see in which stage which enzymes are active in the parental and in the resynthesized lines.

DH lines would have been more effective as the amount of possible recombinations during meiosis could be reduced. The within here used RILs had to be selfed about minimum 5 times to be mostly homozygote. In marker studies some show still heterozygous behavior and recombinations within the genome after meiosis could have taken place.

A comparison of new resynthesized RILs and there progenitors with “old” and agriculturally used *Brassica napus* lines could investigate differences in enzyme and RNA activities. Genotypes that had a high amount of skewed segregating markers and irregularities in the AFLP and SSR analysis could be sequenced and aligned with sequences derived from the “Brassica genome project” to see if and in which regions recombinations took place.

It could also be interesting to calculate the expected amount of fixed heterosis for both newly resynthesized and already used varieties. Than breeders could calculate the gain of heterosis effect they could use by selectively resynthesize new *B. napus* lines.

6 References

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7 Summary

The spontaneous hybridization of related species by combining their genomes (alloploidy) has played a prominent role in plant evolution. Main reasons for the success of allopolyploids are the favourable interactions between loci on homoeologous chromosomes which is similar to the positive interactions between different alleles of the same locus causing classical heterosis in heterozygous genotypes. Those favourable interactions between homoeologous loci should result in an increased performance of allopolyploids compared to their parental species, even in homozygous genotypes. Therefore, such positive epistatic interactions can be called "fixed heterosis". *Brassica napus* (genome constitution AACC) is a very suitable model system to analyze "fixed" heterosis and intergenomic dominance because artificial "resynthesized" lines can easily be developed from diploid parental species *B. rapa* (AA) and *B. oleracea* (CC). It is also possible to produce genotypes of the constitutions AAAC or ACCC. When comparing such genotypes with the mean of their parents (AA and AACC or AACC and CC, respectively), the occurrence of "intergenomic dominance" can also be investigated.

The aim of this project was (i) to analyze the effect of intergenomic dominance for different dosages of the A and C genome in tetraploids and triploids, and (ii) to perform a comparative mapping of QTL involved in fixed heterosis between the parental species *B. rapa* and *B. oleracea* and the allopolyploid.

For the analysis of intergenomic dominance all possible crossings between the diploid and tetraploid homozygous parental lines and the resulting resynthesized rapeseed were performed for two different combinations of the A and C genome – one *B. rapa var oleifera* and one *B. oleracea var. alboglabra*, and one *B. rapa var. trilocularis* and one *B. oleracea var. alboglabra*. Via bud pollinations and for the cross AACCxCCCC additional via embryo rescue seeds were developed. Two replicated trials with two plants per genotype in four randomized blocks and for the embryo rescue trial four randomized blocks with four plants each took place in the greenhouse. After 33 days after sowing or transferring to soil, respectively, the total fresh plant biomass and dry matter was measured.

In the tetraploid comparisons the resynthesized genotype had a higher performance over all trials. The ACCC was once in the seed trial and once in the embryo rescue derived plants significantly better than the tetraploid *B.*

oleracea and better than the mean of both parents. Within the triploid forms the AAC genotypes show a biomass yield that was comparable to the better triploid (AAA). The results indicated that in intergenomic effects exists and in AAC also fixed heterosis occurs. But the amount of the effect depends directly on the genetic material used for the trials and could not be generalized.

The genetic materials for the comparative QTL mapping comprise two homozygous *Brassica rapa* lines (subspecies *trilocularis* and *olifeira*) and two homozygous *Brassica oleracea var. alboglabra* lines. The lines have been selected depending on their detected amount of fixed heterosis in former studies. The resynthesis of those four lines showed a fixed mid-parent-heterosis between 49.9% and 70.5%.

Populations of 150 recombinant inbred lines (RILs) each from crosses between the two *B. rapa and* the two *B. oleracea* lines and a population of 222 lines from a cross between the two resulting synthetic *B. napus* lines are developed. Fixed heterosis is not depending on heterozygosity, and therefore the mapping populations consist of homozygous plants (instead of testcrosses required to analyze QTL for classical heterosis). To identify the contribution of individual genes to the fixed heterosis by QTL mapping, three segregating populations were developed. Two of them in the diploid parental species only segregating for loci in the A and C genome, respectively, and a third one developed from a corresponding allopolyploid which is segregating for loci in both genomes. The RIL were selfed via single seed descent five to six times. The phenotypic data used for the QTL mapping derived from a greenhouse trial where the fresh early plant biomass and dry matter 18 and 20 days after sowing were measured for all lines. Four replications with eight plants per replication were sown following an alpha lattice design. For all measured traits the genotypes of the three populations were significantly different. Also an amount of fixed heterosis of 33.3% up to 48.9% compared to the parental mean occurred for the different traits.

The three populations were analyzed with 28 amplified fragment length polymorphism (AFLP) primer combinations resulting in 276 up to 297 marker points. In the allopolyploid population also 20 single sequence repeat (SSR) primer pairs were used to allow an alignment with genetic maps of former studies. The resulting linkage maps had a size of 1850 cM in *B. rapa* with 10 linkage groups, 1546.1 cM distributed among 15 linkage groups in the *B. oleracea* population and 2373.4 cM on 23 linkage groups for the *B. napus* lines.

The analysis of putative main effect QTL resulted in a total of 29 QTL in the allopolyploid. Eighteen QTL occurred in *B rapa,* eight corresponding with QTL found in the allopolyploid. In *B. oleracea* 30 putative main effect QTL could be observed of which eight correspond with QTL detected in the allopolyploid. Also QTL were detected in all three populations in corresponding regions in the A and C genome.

The QTL mapping for loci involved in epistasis resulted in the localization of 52 epistatic loci pairs where six digenic interactions were between loci showing a significant additive effect. For five loci also a putative main effect QTL was detected.

The result of the current study indicated that it is possible to compare QTL within the different *Brassica* species and there are QTL for fixed heterosis. Although the trait early plant biomass was measured some of the detected QTL are placed near “hot spots” for seed yield and heterosis detected in other studies on the linkage groups N6, N7 and N16.

The interactions between homoeologous chromosomes seem to have an effect that could be called fixed heterosis. This effect is even detectable if only one copy of the allele exists. Further investigations on the mechanisms especially in understanding epistasis are necessary before the benefit could be used in practical breeding.

8 Zusammenfassung

Bei der Evolution der Pflanzen spielte die spontane Hybridisierung verwandter Arten durch Kombination ihres Genoms (Allopolyploidy) eine wichtige Rolle. Einer der Gründe für den Erfolg von Allopolyploiden ist die positive Interaktion zwischen homöologen Genloci, die den positiven Interaktionen zwischen unterschiedlichen Allelen ähnelt, welche zu klassischer Heterosis bei heterozygoten Genotypen führen. Selbst bei homozygoten Genotypen sollten diese positiven Interaktionen zwischen homöologen Loci in einer Mehrleistung der Alloploypolyploiden verglichen mit ihren Elternlinien resultieren. So können diese günstigen epistatischen Interaktionen als „fixierte Heterosis“ bezeichnet werden. *Brassica napus* mit seiner Genomzusammensetzung AACC bietet sich als Modellsystem für Untersuchungen von fixierter Heterosis und intergenomischer Dominanz an, da resynthetisierte Linien einfach aus den diploiden Eltern *B. rapa* (AA) und *B. oleracea* (CC) erstellt werden können. Außerdem ist es möglich auch Genotypen mit der Zusammensetzung AAAC oder ACCC zu produzieren. Indem man diese Genotypen mit dem Mittel ihrer Eltern (AA und AACC, AACC und CC) vergleicht, kann man das Vorkommen von „intergenomischer Dominanz“ untersuchen.

Das Ziel dieser Arbeit war es (i) die intergenomischen Dominanzeffekte für unterschiedliche Anteile von A und C Genom in Tetraploiden und Triploiden zu analysieren und (ii) eine vergleichende QTL Kartierung für fixierte Heterosis zwischen den Elternlinien *B. rapa* und *B. oleracea* und der Allopolyploiden durchzuführen.

Für die Untersuchungen der intergenomischen Dominanz wurden alle zwischen den diploiden und tetraploiden homozygoten Elternlinien möglichen Kreuzungen erstellt. Hierzu wurde in zwei verschiedenen Kombinationen von A und C Genomen gekreuzt: eine *B. rapa var. oleifera* mit einer *B. oleracea var. alboglabra* und eine *B. rapa var. trilocularis* mit einer *B. oleracea var. Alboglabra*. Die Samen wurden durch Knospenbestäubung produziert und für die Kreuzung aus AACCxCCCC wurden zusätzlich mit Hilfe von embryo rescue Pflanzen erstellt. Im Gewächshaus wurden dann mit zwei Wiederholungen mit je zwei Pflanzen in vier randomisierten Blöcken und bei den embryo rescue Pflanzen mit vier randomisierten Blöcken mit jeweils vier Pflanzen Biomasseversuche durchgeführt. Dreiunddreißig Tage nach der Aussaat, bzw. nach dem Überführen in Erde, wurden die überirdische Biomasse und das Trockengewicht bestimmt.

Im Vergleich zwischen den Tetraploiden wiesen die Resynthesen über alle Versuche das höchste Gewicht auf. Die ACCC-Kombinationen waren einmal beim normalen Aussatversuch und einmal bei den durch embryo rescue erhaltenen Pflanzen signifikant besser als die tetraploide *B. oleracea* und besser als das Mittel beider Eltern. Bei den diploiden Formen wiesen die AAC Genotypen einen mit dem besseren Triploid AAA vergleichbaren Biomasseertrag auf. Diese Ergebnisse weisen darauf hin, dass ein intergenomischer Effekt auftritt, ebenso wie fixierte Heterosis, die in den AAC Genotypen zu beobachten war. Das Ausmaß der Effekte war jedoch stark vom ausgewählten genetischen Material abhängig und kann so nicht verallgemeinert werden.

Das genetische Material für die vergleichende QTL-Kartierung bestand aus zwei homozygoten *Brassica rapa* Linien (subspecies *trilocularis* und *olifeira*) und zwei homozygoten *Brassica oleracea var. alboglabra* Linien. Die Linien wurden anhand ihres in früheren Studien gemessenen Anteils an fixierter Heterosis ausgewählt. Die Resynthesen dieser vier Linien wiesen eine fixierte mittlere Elternheterosis zwischen 49.9% und 70.5% auf.

Zu jeder Kreuzung zwischen den zwei *B. rapa* und den zwei *B oleracea* Linien wurden jeweils 150 rekombinante Inzucht Linien (RILs) erstellt und für die Kreuzung zwischen den zwei resultierenden Resynthesen wurden 222 Linien erstellt. Da fixierte Heterosis nicht von der Heterozygotie abhängt, bestehen die Kartierungspopulationen aus homozygoten Pflanzen (anstatt Testkreuzungen, die bei der Analyse von klassischer Heterosis benötigt werden). Um den Beitrag einzelner Gene zur fixierten Heterosis via QTL-Kartierung zu bestimmen, wurden drei Populationen entwickelt. Zwei davon in den diploiden Elternlinien, die je nur für die Loci im A und C aufspalten und eine dritte, die für beide Loci segregiert. Die RIL wurden durch Einzelsamennachkommenschaften fünf- bis sechsmal geselbstet. Die phänotypischen Daten, die für die QTL-Kartierung benötigt werden stammen, aus einem Gewächshausversuch, wobei die frühe Frischbiomasse und die Trockenmasse 18 und 22 Tage nach der Aussaat bestimmt wurden. Vier Wiederholungen mit acht Pflanzen je Wiederholung wurden hierzu in einem Alpha-Lattice-Design ausgelegt. Für alle untersuchten Eigenschaften waren die Genotypen der drei Populationen signifikant verschieden. Außerdem konnte ein Ausmaß an fixierter Heterosis von 33.3% bis 48.9% im Vergleich zum Elternmittel für die verschiedenen Merkmale bestimmt werden.

Die drei Populationen wurden mit 28 Amplified Fragment Length Polymorphism (AFLP) Markern untersucht, wobei zwischen 276 und 297

Markerdatenpunkte erhalten worden sind. In der Allopolyploiden wurden außerdem 20 Single Sequence Repeats (SSR) Primer benützt, um ein Alignment mit Genkarten aus anderen Studien zu ermöglichen. Die daraus resultierenden Kopplungskarten hatten eine Größe von 1850 cM in *B. rapa* bestehend aus 10 Kopplungsgruppen, 1546.1 cM und 15 Kopplungsgruppen in der *B. oleracea* Population und 2373.4 cM auf 23 Kopplungsgruppen bei den *B. napus* Linien.

Bei der Analyse der Haupteffekt-QTL konnten in der Allopoplyploiden 29 QTL gefunden werden. Achtzehn QTL wurden in *B rapa* detektiert, acht davon korrespondierten mit QTL die in der Allopolyploiden gefunden wurden. In *B. oleracea* konnten 30 Haupteffekt-QTL beobachtet werden, wovon ebenfalls acht auch in der Allopolyploiden auftauchten. Außerdem konnten QTL detektiert werden, die in allen drei Populationen in den korrespondierenden Regionen im A und C Genom auftauchten.

Die QTL-Kartierung für epistatische QTL resultierte in 52 epistatischen Locipaaren, wobei sechs der digenischen Interaktionen zwischen Loci waren, die einen signifikanten Additiveffekt aufwiesen. Für fünf Loci wurde ebenfalls ein Haupteffekt-QTL detektiert.

Anhand des Ergebnisses dieser Studie konnte nachgewiesen werden, dass es möglich ist, QTL innerhalb verschiedener *Brassica*-Spezies zu vergleichen und dass es QTL für fixierte Heterosis gibt. Obwohl als Merkmal die frühe Biomasse gewählt wurde, konnten einige der detektierten QTL nahe bei “hot spots” auf N6, N7 und N16 für Ertrag und Heterosis aus anderen Forschungsgruppen lokalisiert werden.

Die Interaktionen zwischen homöologen Chromosomen scheinen einen Effekt zu haben, den man als fixierte Heterosis bezeichnen kann. Dieser Effekt ist bereits dann nachweisbar, wenn nur eine Kopie eines Allels vorliegt. Weitere Forschungsarbeiten zu diesem Mechanismus und besonders zum Verständnis von Epistasie sind notwendig, bevor dieser Vorteil auch in der praktischen Züchtung genutzt werden kann.

9 Appendix

A

CCC	ACC	AACC	ACCC	AAAC	CC	AAAA	ACC	AA	CCCC	AAC
AA	AAA	AAC	AAAA	CCCC	AAA	CC	CCC	AACC	AAAC	ACCC
ACC	CCC	AAAC	ACCC	AACC	AAA	AACC	AA	ACCC	AAC	AAA
AAC	CCCC	AA	AAAA	CC	ACC	CCCC	CCC	AAAA	CC	AAAC

B

AAAA	CCC	ACA	ACCC	AAA	AA	ACC	ACA	AAA	AACC	AA
ACAA	ACC	CC	AACC	CCCC	CC	ACCC	CCC	CCCC	ACAA	AAAA
AA	CCC	ACA	AACC	CCC	AAA	ACCC	AAAA	AA	ACAA	CC
ACCC	CC	AAAA	ACC	ACAA	CCCC	AACC	AAA	ACA	ACC	CCC

C

AAA	AAAC	AACC	AAC	CCC	ACCC	ACCC	CCCC	AAAC	AA	CCC
CC	CCCC	AA	AAAA	ACC	AAAA	AACC	ACC	AAC	CC	AAA
AAAA	CCC	AAAC	CC	ACC	AAA	AAC	CCCC	AACC	AA	ACC
CCCC	AAC	ACCC	AA	AACC	AAAC	AAA	ACCC	CC	AAAA	CCC

D

AAAA	ACA	ACAA	AACC	AA	AAA	CCCC	CC	ACCC	ACA	ACC
ACC	CC	CCCC	ACCC	CCC	AAA	ACAA	CCC	AA	AACC	AAAA
AAAA	ACC	ACCC	CCCC	ACA	AACC	CC	AAA	ACCC	ACAA	CCC
ACAA	CC	AA	CCC	AAA	AA	AACC	ACA	ACC	AAAA	CCCC

Figure 15: Randomization for the biomass trials; A_2A_2 C_4C_4 combinations repeat one (A) repeat two (B) and for combinations of A_5A_5 C_6C_6 repeat one (C) and two (D)

A

		I						II			
AACC 1	2	**ACCC** 4	3	**CCCC** 3	4	**CCCC** 2	3	**ACCC** 2	4	**AACC** 2	4
3	4	2	1	2	1	4	1	1	3	3	1
CCCC 1	4	**AACC** 4	1	**ACCC** 3	4	**CCCC** 4	2	**ACCC** 4	1	**AACC** 2	1
3	2	2	3	1	2	1	3	3	2	4	3
		III						IV			

B

		I						II			
CCCC 1	3	**ACCC** 4	1	**AACC** 4	1	**AACC** 1	3	**CCCC** 2	3	**ACCC** 2	1
2	4	3	2	2	3	2	4	1	4	4	3
ACCC 4	2	**CCCC** 2	4	**AACC** 2	3	**ACCC** 1	4	**CCCC** 4	1	**AACC** 4	1
3	1	1	3	4	1	2	3	2	3	2	3
		III						IV			

Figure 16: Randomized block design for the biomass trials of the embryo rescue plants. Design for A_2A_2 C_4C_4 combinations (A) and the combinations of A_5A_5 C_6C_6 genotypes (B)

Table 9: AFLP primer combinations

Name	Code	Colour
M47	GAT GAG TCC TGA GTA ACA A	
M49	GAT GAG TCC TGA GTA ACA G	
M53	GAT GAG TCC TGA GTA ACC G	
M55	GAT GAG TCC TGA GTA ACG A	
M57	GAT GAG TCC TGA GTA ACG G	
M59	GAT GAG TCC TGA GTA ACT A	
M61	GAT GAG TCC TGA GTA ACT G	
E32	CTG CGT ACC AAT TCA AC	(PET)
E33	CTG CGT ACC AAT TCA AG	(NET)
E34	CTG CGT ACC AAT TCA AT	(VIC)
E35	CTG CGT ACC AAT TCA CA	(6-FAM)
E36	CTG CGT ACC AAT TCA CC	(PET)
E37	CTG CGT ACC AAT TCA CG	(PET)
E38	CTG CGT ACC AAT TCA CT	(NET)
E40	CTG CGT ACC AAT TCA GC	(6-FAM)
E42	CTG CGT ACC AAT TCA GT	(VIC)
E44	CTG CGT ACC AAT TCA TC	(VIC)
E45	CTG CGT ACC AAT TCA TG	(6-FAM)

M: Mse primer; E Eco primer;

Table 10: SSR primer pairs; LG linkage group and*Position in cM in the linkage map of Radoev (2007)

Marker	LG	Position*
*CB10206	1	28,1
*CB10097	1	87,7
*CB10540	2	32,5
*CB10347	4	22,8
*CB10493a	4	73,8
*CB10051	5	55,6
*CB10030	6	0,0
*CB10613	6	57,0
*CB10439	7	0,0
*CB10003	8	24,2
*BRAS039	8	36,4
*BRAS020	9	0,0
*BRAS010b	9	13,6
*CB10373b	9	48,0
*MR156	10	23,2
*CB10284	10	29,4
*CB10109b	10	30,4
*CB10265	10	85,3
*MR230	9	85,8
*CB10159	1	27,8
*CB10189	1	32,9
*CB10167	1	38,3
*BRAS002b	3	96,9
*MR119	5	53,4
*CB10121	6	47,6
*CB10587	11	0,0
*CB10369	11	32,1
*CB10316	12	6,2
*CB10600	12	23,8
*BRAS065	13	36,7
*BRAS076	13	82,0
*CB10513	13	157,4
*MR36	14	21,9
*MR229	14	26,1
*CB10611	15	42,2
*BRAS052	15	43,2
*CB10065	15	56,8
*CB10435	15	64,1
*CB10027	15	71,5
*CB10632	16	37,6
*CB10213	16	62,1
*CB10278	16	65,9
*CB10234	16	88,1
*CB10034	17	38,3
*CB10433	17	58,1
*CB10101	17	73,9
*CB10028	18	26,1
*CB10504	18	40,7
*CB10530	19	87,3
*CB10288	19	98,8

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