Rapeseed as a model to analyse "fixed heterosis" in alloploid plants

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Abstract

We propose an experimental approach to quantify "fixed heterosis" in resynthesised *Brassica napus* and to detect loci contributing to "fixed heterosis" via comparative QTL mapping in *B. napus* and its parental species *B. rapa* and *B. oleracea*. To identify the contribution of individual genes to the fixed heterosis by QTL mapping, three segregating RIL populations were developed. The marker analysis will be performed with AFLPs and SSR marker allowing a comparison with results of groups working with *Brassica* and *Arabidopsis.*

Key words: fixed heterosis, allopolyploids, *Brassica,* QTL analysis

Introduction

The spontaneous hybridisation of related species by combining their genomes (alloploidy) has played a prominent role in plant evolution. A main reason for the success of allopolyploids are the favourable interactions between genes on their homeologous chromosomes which is similar to the positive interactions between different alleles of one gene causing classical heterosis (Shull, 1908) 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 1). Therefore, such positive epistatic interactions can be called "fixed heterosis" (Abel et al., 2005). *Brassica napus* (genome constitution AACC) is a very suitable model system to analyse "fixed" heterosis, because artificial "resynthesized" lines can easily be developed from diploid parental species *B. rapa* (AA) and *B. oleracea* (CC).

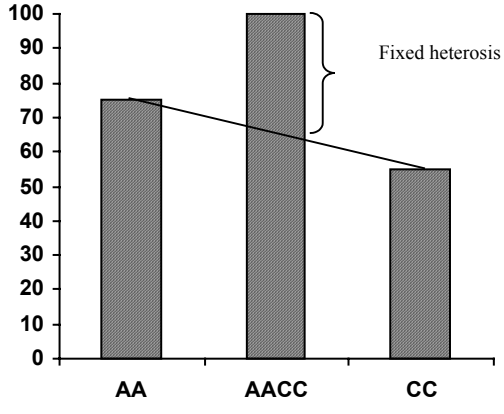

Figure 1: Schematic diagram of the effect of fixed heterosis (relativ performance)

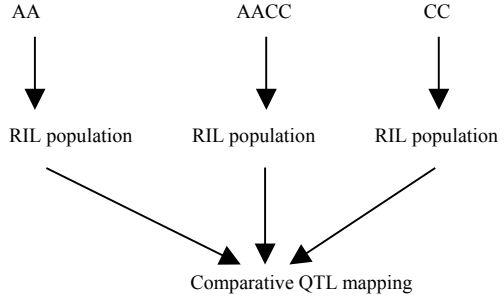

Figure 2: Detection of fixed heterosis into individual QTL

Material

The genetic materials comprise one *Brassica rapa var. trilocularis* line (RO18 = A_1) from the John Innes Center, one *Brassica rapa var. olifeira* line (6748-1430 = A_2) from Agri Food Canada and two *Brassica oleracea var. alboglabra* lines (A12 = C_3 and S2C3-4-1 = C_4) (John Innes Center and Crucifer Genet. Cooper). All lines were double haploids or highly inbred lines. For a detailed description of the material see Abel et al. (2005). The lines have been selected depending on their detected amount of fixed heterosis. The resynthesis of this four lines showed a fixed mid-parent-heterosis between 49,9% and 70,5%.

Recombinant inbred lines (RILs) from a cross between the two *B. rapa*, the two *B. oleracea* and the resulting synthetic *B. napus* line are developed as shown in Figure 2. In the parental lines (A_1A_2, C_3C_4) a sample of 150 RILs each will be genotyped. For a comparable detection power for the QTL in the amphidiploid species an amount of 300 RILs will be used. Due to the polyploidisation the genetic variance explained by the QTL will be higher in the parental RILs than the genetic variance explained in the amphidiploid species. Therefore more lines (N=300) have to be analysed in the resynthesized *B. napus* to reach a comparable detection intensity.

QTL mapping

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).

To identify the contribution of individual genes to the fixed heterosis by QTL mapping, three segregating populations were developed (Figure 3). 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.

In this study we will use AFLP and SSR markers. AFLPs (Vos et al., 1995) are a cost effective and fast technique for detecting polymorphisms in DNA and for developing a framework map. In addition some SSR markers that are distributed equally over the genome are needed to align the results with other linkage maps. After this first approach more SSRs will be selected supplementary that are more closely linked to "interesting regions".

In former studies saturated mapping of microsatellite markers in *B. oleracea*, as well as in *B. campestris and B. napus*, could be successfully used to reveal the homoeologous relationships between *B. napus* and its progenitor genomes (Saal et al., 2001). So SSR loci are well conserved among various *B .rapa* accessions, and most can be applied to related species such as *B. oleracea*, *B. napus*, and *A. thaliana* with high accuracy (Suwabe et al., 2006). A linkage map based on molecular markers that would allow discrimination between homologous and homeologous regions is required for a comparative analysis in *Brassica*.

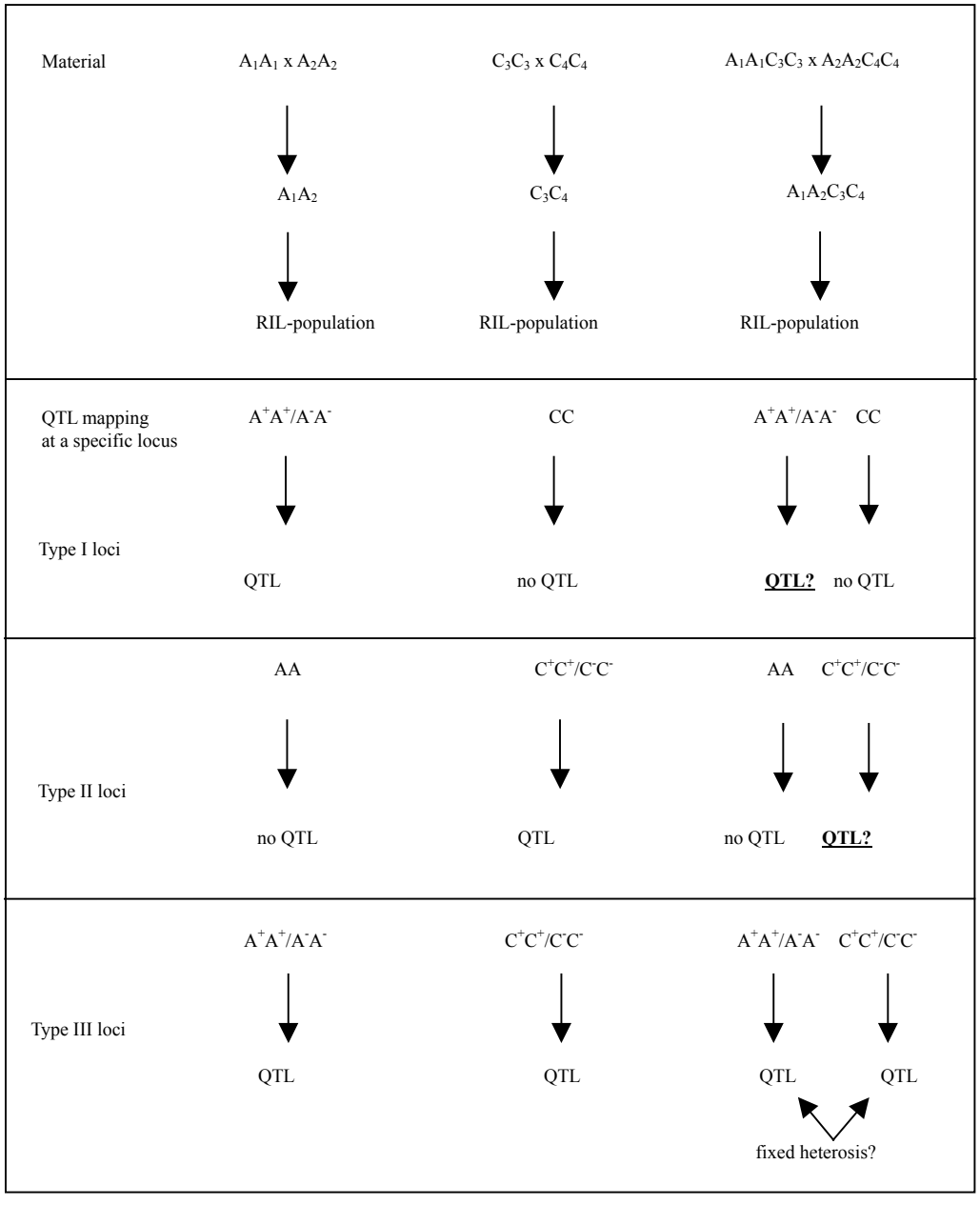

Figure 3: Principles of QTL mapping to analyse fixed heterosis (for explanation see text)

For the observed QTL three types can be classified as follows (Figure 3):

I QTL which are detected in the A genome but not at the corresponding position in the C genome;
II QTL which are detected in the C genome but not at the corresponding position in the A genome;
III QTL which are detected both in the A and C genome at corresponding positions.

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 can be modified 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 (meaning that A^+A^+CC and A^-A^-CC have the same performance). 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 (Table 1), because ($A^+A^+C^-C^-$ + $A^-A^-C^+C^+$) should have a higher performance than ($A^+A^+C^+C^+$ + $A^-A^-C^-C$).

Table 1: Possible allele combinations in the allopolyploid at two homeologous loci and the expected occurrence of fixed heterosis

Genotype	Fixed heterosis
$A^+A^+C^+C^+$	no
$A^-A^-C^-C^-$	no
$A^+A^+C^-C^-$	yes
$A^-A^-C^+C^+$	yes

Because homozygous lines are used, only additive effects and additive×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 the genetic variance of the homozygous RIL populations of the parental lines can be defined as $\Sigma\ a_A^2$ and $\Sigma\ a_C^2$. Respectively, the genetic variance of the allopolyploid genome is defined as $\Sigma\ a_A^2 + \Sigma\ a_C^2$. In case of deviation from the expected genetic variance in the allopolyploid situation this would be an evidence for epistatis. If fixed heterosis exists it is to be expected that the variance among the RILs of the alloplolyploid is larger than the sum of the variance of the diploid RILs.

Outlook

The expectations described above are only valid if the effect of a negative allele in one genome can be completely compensated by a positive allele in the other genome, otherwise the QTL will not completely disappear but will have a smaller effect. Moreover, not all QTL contributing to fixed heterosis can be detected but only those QTL which segregate at least in one of the two genomes. Nevertheless this approach will allow 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 will be possible to compare these results with the results of QTL mapping in other groups working on *Brassica* and on *Arabidopsis*.

Additionally phenotypic data will be evaluated via biomass measurement of all genotyped RILs. The trait biomass has been chosen because of the expected difficulties in the flowering behavior, fertility and seed development especially due to the high inbreeding generation of the tested lines. By analysing the data from the biomass trials detected QTL which influence fixed heterosis can be identified.

As to our knowledge, this is a new approach still not experimentally tested. The experiments already started and we hope to present interesting results soon.

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References

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