# Quantitative trait loci influencing heterosis for seedling biomass and seed yield in oilseed rape

**Panjisakti Basunanda, Maen Hasan, Tobias Spiller, Rod Snowdon, Wolfgang Friedt**

*Department of Plant Breeding, Research Centre for BioSystems, Land Use and Nutrition,*
*Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany*
Email: panjisakti.basunanda@agrar.uni-giessen.de

**Abstract**

Understanding the genetic basis of early developmental and biomass traits in oilseed rape (*Brassica napus* var. *oleifera*) and investigation of possible relationships to seed yield could be potentially useful with respect to hybrid breeding. One interesting aspect that to date has not been fully elaborated is whether heterosis for seedling traits can be related to heterosis for seed yield. In this study we have mapped quantitative trait loci (QTL) influencing heterosis for morphological traits in oilseed rape seedlings grown under controlled conditions. Our aim is to compare these loci to QTL influencing heterosis for seed yield in field experiments in the same mapping population.

**Key words:** heterosis, quantitative trait locus, seed yield, oilseed rape.

## Introduction

Investigation of the genetic basis of early developmental and biomass traits in oilseed rape (*Brassica napus*) and possible relationships to seed yield could be potentially useful with respect to hybrid breeding. Trials to select crosses leading to higher yield are costly, thus detecting seedling traits with correlations to yield or yield heterosis could be valuable in reducing the cost. Diepenbrock (2000) suggested that the number of pods per plant is decisive for seed yield in oilseed rape, and that pod length may be used as selection variable in breeding since it is simple to measure.

The relationship between heterosis for early developmental traits and seed yield in oilseed rape has not been investigated to date. In this respect, genetic mapping of loci involved in such traits could be a potentially useful tool to locate chromosome regions that are responsible for heterosis. Colocation of QTL relevant to heterosis for yield and early developmental traits could potentially identify closely-linked markers to significant loci that could assist in breeding efforts. El-Lithy et al. (2004) performed QTL analysis for early physiological traits in *Arabidopsis thaliana*, and genetic mapping was also used to elucidate the genetic control of heterosisin rice (Zhao 1999), wheat (Liu et al. 1998) and maize (Hoecker et al. 2006). However to our knowledge investigations of heterosis for seedling developmental traits and their correlation to yield heterosis have not been published in oilseed rape until now. In this study we have mapped quantitative trait loci (QTL) influencing heterosis for morphological traits in oilseed rape seedlings grown under controlled conditions. Our aim is to compare these loci to QTL influencing heterosis for seed yield in field experiments.

## Material and Method

A set of doubled-haploid (DH) lines was developed from a cross between the 00-quality winter oilseed rape variety 'Express' and the high erucic acid, high glucosinolate inbred line 'V8'. This DH population was used to develop a set of test hybrids by controlled crossing of all DH lines with the male-sterile tester 'MSL-Falcon'. The material was subjected to a greenhouse and a field trial including the parental lines as controls. Seedling dry weight, leaf area and seedling height data were collected as indicators for seedling biomass heterosis at 17 and 28 days after sowing (*das*) from a triple-replicated greenhouse experiment. Field trials of the same materials were conducted in 2004-2005 at two locations to estimate yield and yield heterosis. All phenotypic data were analysed using PROC MIXED available in SAS v. 8.1.

A genetic map comprising 114 SSR and 155 AFLP markers was generated from the DH population and used to locate relevant QTL of the investigated traits. The genetic map was made using JoinMap® v. 3.0 (van Oijen and Voorrips 2001) and consists of 19 linkage groups covering a total length of 1791.2cM. QTL analysis was performed using PLABQTL version 1.2 (Utz and Melchinger 2003) which applies regression methods to detect significant QTL using composite interval mapping. A LOD threshold of 3.0 was used as the significance level for detection of QTL.

## Results

Quantitative trait loci (QTL) for *per se* performance with additive effects were obtained using the data from the DH lines, while QTL for heterosis in the respective traits could be localised based on the differences between test hybrid and mid-parent data in the respective experiments.

A total or 13 significant QTL were localised for all of the traits investigate, of which four QTL are related to heterosis and nine to *per se* performance (Table 1). Three QTL for heterosis in seedling traits were detected along with one QTL for seed yield heterosis. A strong additive QTL on chromosome N17 was found to have a significant effect on seedling dry weight at

17 days after sowing (das), however at 28 das no QTL was found at this position and a different locus on chromosome N10 had the main effect on this trait. Two different QTL were detected with significant effects on heterosis for hypocotyl length at 17 das and stem length at 28 das. This suggests a shift in the genetic control of heterosis for seedling development during the first four weeks after germination. Only one QTL for heterosis in seed yield was detected, and this QTL was not in the same position as the main QTL for *per se* yield. The QTL for seed yield heterosis did not colocalise with the QTL for seedling biomass heterosis.

**Table 1. Significant QTL for seedling biomass traits, yeed yield and heterosis in the respective traits.**

| Trait | QTL position | Interval (cM) | LOD | Part. $R^2$ | Additive effect | Heterosis |
|---|---|---|---|---|---|---|
| Root dry weight 17 *das* | N17: 48 cM | 44 - 52 | 6.58 | 6.3 | 0.76 | - |
| Leaf dry weight 17 *das* | N17: 46 cM | 42 - 52 | 4.26 | 9.1 | 2.16 | - |
| Leaf area 17 *das* | N17: 48 cM | 40 - 54 | 3.11 | 9.1 | 0.64 | - |
| Hypocotyl length 17 *das* | N5: 86 cM | 80 - 86 | 3.57 | 4.8 | - | 2.30 |
| Shoot dry weight 28 *das* | N10: 70 cM | 62 - 74 | 5.20 | 8.2 | -5.95 | - |
| Leaf dry weight 28 *das* | N10: 70 cM | 60 - 74 | 4.29 | 8.3 | -4.24 | - |
| Leaf area 28 *das* | N6: 36 cM | 30 - 44 | 3.03 | 4.4 | - | 1.64 |
| Stem length 28 *das* | N7: 38 cM | 32 - 42 | 4.38 | 11.6 | -2.10 | - |
| | N12: 74 cM | 70 - 76 | 6.17 | 8.8 | -1.71 | - |
| | N17: 70 cM | 66 - 76 | 3.70 | 11.5 | -2.02 | - |
| | N18: 2 cM | 0 - 8 | 3.22 | 3.8 | - | 0.94 |
| Seed yield | N5: 32 cM | 20 - 44 | 3.37 | 3.8 | - | 2.05 |
| | N7: 26 cM | 24 - 30 | 3.48 | 6.1 | 1.42 | - |

## Discussion

The co-location of QTL for seedling root and leaf dry weight and leaf area, along with a corresponding correlation among these traits, indicate a combined control of these traits during early seedling development. Interestingly, however, different main loci were found to influence these traits at the two different stages of seedling development. El Lithy et al. (2004) found a strong correlation between seed weight and some early morphological traits in *A. thaliana* seedlings, which also diminished at later stage of development. This shift in genetic control of the developmental process appeared to occur during the first four weeks of seedling development.

In this work we did not find any co-location between additive QTL for *per se* trait values in the DH lines and QTL for heterosis in the corresponding traits. Most of the QTL had only a small contribution to the total phenotypic variation. This indicates the complex character of the traits being examined. Furthermore, no co-location was observed between QTL for early developmental traits and for yield or for heterosis in these traits. However further data may increase the resolution of the QTL analysis to enable us to detect additional QTL with effects on different traits. Ultimately we are interested in identifying genes involved in regulation of heterosisin oilseed rape. Birchler et al. (2003) gave an insight into the molecular basis of heterosis in which they described the possibility that the combination of different alleles could produce interactions that cause gene expression in the hybrid to deviate relative to the midparent predictions. This may cause up-regulation of many housekeeping genes that are associated with increases in performance. Relating marker-based QTL analysis with expression of known genes, for example through expression-QTL (eQTL) analysis, could be valuable in deciphering the genetic control of heterosis and obtaining information of use for controlled use of this phenomenon for breeding purposes.

## Summary

Evidence was obtained that biomass heterosis can be measured at early developmental stages in oilseed rape. It appears that the genetic control of heterosis changes in different developmental stages. One significant QTL for seed yield heterosis could be located. The investigations are currently being continued with a different set of test hybrids and compared with data from a different mapping population. Ultimately we hope to gather information which will help in determining the fundamental genetic mechanisms underlying developmental and yield heterosis in *B. napus*.

## Acknowlegements

This work is being carried out within the DFG special research program SPP 1149 ”Heterosis in Plants” in collaboration with the University of Göttingen. PB received additional financial support from DAAD. The authors particularly thank Jörg Schondelmaier for valuable assistance with the genetic mapping, along with Liane Renno, Mechthild Schwarte and Martin Sondermann for excellent technical assistance. Non-public SSR markers were kindly provided by Saatenunion Resistenzlabor GmbH and Wolfgang Ecke from University of Göttingen.

## References

Birchler, J.A., D.L. Auger, N.C. Riddle. 2003. In search of the molecular basis of heterosis. The Plant Cell 15: 2236-2239.

Diepenbrock W. 2000. Yield analysis of winter oilseed rape (Brassica napus L.): a review. Field Crop Res. 67: 35-49.

El Lithy M E., E.J.M. Clerkx, G.J. Ruys, M. Koornneef, and D. Vreugdenhil. 2004. Quantitative Trait Locus Analysis of Growth-Related Traits in a New Arabidopsis Recombinant Inbred Population. Plant Phys. 135: 444-458.

Hoecker N., B. Keller, H.-P. Piepho, F. Hochholdinger 2006. Manifestation of heterosis during early maize (Zea mays L.) root development. Theor Appl Genet (2006) 112: 421–429.

Liu, Z.-Q., Y. Pei, Z.-J. Pu. 1999. Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, Triticum aestivum L. Plant Breeding 118: 119-123

van Oijen, J.W. and R.E. Voorrips 2001. JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, Netherlands.

Utz, H.F. and A.E. Melchinger. 2003. PLABQTL, a computer program to map QTL. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Zhao M. F., X. H. Li , J. B. Yang , C. G. Xu, R. Y. Hu , D. J. Liu, Q. Zhang. 1999. Relationship between molecular marker heterozygosity and hybrid performance in intra- and inter-subspecific crosses of rice. Plant Breeding 118: 139-144.