QTL mapping in Rapeseed (*B. napus* L.) and analysis of interactions between QTL effects and nitrogen fertilization

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Introduction

Most agronomically important traits show a continuous range of trait expressions in segregating populations, making it difficult to locate and characterize the genetic factors involved in the inheritance of these traits. Moreover, trait expression in individual genotypes often is subject to interactions with the environment, indicating that the effects of the genes controlling these traits are affected by environmental conditions. A new approach to QTL mapping, integrating QTL detection and localization with an analysis of QTL x environment interactions, was used to map QTL for a range of agronomically interesting traits and to analyze the interactions of these QTL with different levels of nitrogen fertilization.

Materials and Methods

The QTL were mapped in a segregating doubled haploid population derived from microspores of F_1 plants of a cross between doubled haploid lines of the winter rapeseed varieties 'Mansholt´s Hamburger Raps' and 'Samourai'. For QTL mapping a framework map of 185 well spaced marker loci covering 1739 cM (Haldane) on 20 linkage groups was derived from a RFLP map previously developed in the doubled haploid population (Uzunova et al. 1995).

In each of the growing years 1998/99 and 1999/00 142 lines of the doubled haploid population were grown at two locations in Göttingen in two replications with two different Nitrogen fertilizer treatments: unfertilized (N_0) and 240 kg/ha Nitrogen (N_1). Per treatment, year and location two replications were grown in double rows (2,5 m, 40 plants per row).

A total of 13 traits (Tab. 1) was evaluated in the field trials. Estimation of variance components was done with the program PLABSTAT (Utz 1997). For QTL mapping and the analysis of QTL interactions with nitrogen fertilization the program QTLMapper was used. This program implements a Mixed Model Composite Interval Mapping (MCIM) approach which allows to simultaneously map QTL and analyze QTL x environment interactions (Wang et al. 1999). For each doubled haploid line and fertilizer treatment trait means were calculated from all years and locations and used for QTL mapping. The fertilizer treatments were entered as two different environments. For detection of significant QTL effects a threshold of P≤ 0.005 was applied.

Results

A total of 57 QTL could be mapped for 13 traits (Tab. 1). The cumulative main effects of the mapped QTL explained between 28.1% and 88.2% of the genetic variance of the respective traits. Only seven traits showed significant (P≤ 0.1) interactions between genotypes and nitrogen fertilization with interaction variances on the average about one tenth of the respective genetic variances. For four of these traits it was possible to assign at least part of the interactions to individual QTL. The strongest QTL interaction effects were observed for protein and oil content.

The number of QTL per trait ranged from 3 to 6 with a large variation in the effects of individual QTL. For protein and oil content, for example, 5 and 6 QTL could be mapped, respectively, with main effects explaining from 3% to 15.2% and 4.1% to 40.3% of the genetic variance of the respective trait (Tables 2, 3). Only a minority of QTL showed significant (P≤ 0.005) interactions with nitrogen fertilization. For protein and oil content only 2 and 1 QTL were found, respectively, but these QTL showed interaction effects in the same order of magnitude as the main effects.

Protein and oil content were negatively correlated with a correlation coefficient of r= -0.653 (P≤ 0.005). A comparison of positions and effects of the QTL mapped for oil and protein content (Table 4) showed 4 of the 5 QTL affecting protein content to be located on linkage groups that also harbor QTL for oil content. For three of these QTL pairs the estimated effects had opposite signs, meaning that with respect to parental origin the alleles of the QTL for protein content decreasing protein content were paired with the alleles of the QTL for oil content increasing oil content and vice versa. Only one QTL pair, on linkage group 5, is closely linked, probably representing the same gene. The other three pairs are far apart on their respective linkage groups, indicating that here the QTL for protein and oil content represent different genes.

Discussion

In the doubled haploid population analyzed significant genotype interactions with nitrogen fertilization were small compared to genetic effects. Nevertheless, using a QTL mapping approach with an integrated analysis of QTL x environment interactions, for four traits substantial parts of these interactions could be assigned to individual QTL. Interestingly, only a small number of QTL contributed to the interactions. For protein and oil content the interaction effects of these QTL were of the same order of magnitude as the main effects.

In the doubled haploid population protein and oil content showed a strong negative correlation. This could be expected since oil and protein content are the main constituents of the rapeseed seed. If one is increased the other has to be decreased. Accordingly, QTL for oil content should also have some effect on protein content and vice versa, as is probably the case on linkage group 5. Surprisingly, our results indicate that linkage between QTL for oil and protein content with the corresponding parental alleles having opposite effects on the two traits may also contribute to the negative correlation.

Literature

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Table 1: Genetic variance, genotype x nitrogen interaction variance, number of mapped QTL and variances explained by the respective QTL effects for 13 traits

Trait	V_G	V_{GN}	QTL no.	VE_{main} [%]	$VE_{QTL\ x\ N}$ [%]
Vigor	1.00 **	0.00	6	51.5	n.e.
Begin of flowering	8.34 **	0.03	5	47.2	n.e.
End of flowering	2.22 **	0.01	4	57.6	n.e.
Duration of flowering	4.67 **	0.05	5	67.6	n.e.
Chlorophyll content at beg. of flow.	4.15 **	1.04 **	5	47.0	20
Chlorophyll content at end of flow.	5.12 **	0.89 **	4	39.9	28
Plant height at end of flowering	72.43 **	2.51 **	3	30.1	0
Pod length	0.14 **	0.01 *	3	28.1	0
Number of seeds per pod	1.82 **	0.38 *	3	33.0	0
Thousand kernel weight	0.09 **	0.00	4	47.8	n.e.
Oil content	2.77 **	0.05 +	6	81.6	155[1]
Protein content	0.87 **	0.07 *	5	39.6	130[1]
Total GSL content	206.15 **	0.90	4	88.2	n.e.

V_G: genetic variance; V_{GN}: variance of genotype interactions with nitrogen fertilization level; VE_{main}: genetic variance explained by main additive effects of mapped QTL;$VE_{QTL\ x\ N}$: interaction variance explaind by QTL x nitrogen level interaction effects;
n. e.: not estimated; +, *, **: significant at P≤ 0.1, P≤ 0.05, P≤ 0.01;
[1]values larger than 100% are due to experimental error leading to an underestimation of genotype x nitrogen interaction variance and/or an overestimation of QTL effects.

Table 2: QTL for protein content and associated effects

QTL	LG[1]	Posit.[2] [cM]	A[3] [%]	EV_{Main}[4] [%]	Prob.[5]	$A_{QTL\ x\ N_0}$[6] [%]	$EV_{QTL\ x\ N}$[7] [%]	Prob.[5]
PC1	2	69.6	-0.216	5.4	0.000	0.083	0.0	0.105
PC2	5	19.9	-0.293	9.9	0.000	-0.254	99.0	0.004
PC3	11	59.3	0.231	6.1	0.002	-0.004	0.0	0.882
PC4	14	93.4	0.162	3.0	0.000	0.142	31.0	0.001
PC5	15	53.9	0.364	15.2	0.000	-0.111	0.0	0.013

[1]Linkage group; [2]QTL position, distance from first marker in linkage group; [3]Additive main effects in percent protein content, effect of replacing a 'Samourai' allele by an allele of 'Mansholt'; [4]Percentage of genetic variance explained by main effects; [5]Significance of QTL effects; [6]Additive interaction effects in percent protein content of N_0 treatment, interaction effects in N_1 are of the same absolute size but with opposite sign; [7]Percentage of genotype x nitrogen fertilizer variance explained by interaction effects

Table 3: QTL for oil content and associated effects

QTL	LG[1]	Posit.[2] [cM]	A[3] [%]	EV_{Main}[4] [%]	Prob.[5]	$A_{QTL\ x\ E}$[6] [%]	$EV_{QTL\ x\ E}$[7] [%]	Prob.[5]
OC1	5	16.1	0.506	9.2	0.000	-0.029	0.0	0.532
OC2	6	56.4	1.056	40.3	0.000	0.152	0.0	0.037
OC3	11	18.3	-0.426	6.6	0.000	0.177	0.0	0.016
OC4	12	124.0	0.664	15.9	0.000	0.128	0.0	0.095
OC5	14	28.2	0.392	5.5	0.000	0.279	155.0	0.000
OC6	15	16.0	-0.335	4.1	0.000	0.013	0.0	0.882

[1]Linkage group; [2]QTL position, distance from first marker in linkage group; [3]Additive main effects in percent oil content, effect of replacing a 'Samourai' allele by an allele of 'Mansholt'; [4]Percentage of genetic variance explained by main effects; [5]Significance of QTL effects; [6]Additive interaction effects in percent oil content of N_0 treatment, interaction effects in N_1 are of the same absolute size but with opposite sign; [7]Percentage of genotype x nitrogen fertilizer variance explained by interaction effects (values larger than 100% are due to experimental error leading to an underestimation of genotype x nitrogen interaction variance and/or an overestimation of QTL effects).

Table 4: Comparison of positions and effects of QTL for protein and oil content

Protein content				Oil content			
QTL	LG.[1]	Posit.[2] [cM]	A_{main}[3] [%]	QTL	LG.[1]	Posit.[2] [cM]	A_{main}[3] [%]
PC1	2	69.6	-0.216		-	-	-
PC2	5	19.9	-0.293	OC1	5	16.1	0.506
	-	-	-	OC2	6	56.4	1.056
PC3	11	59.3	0.231	OC3	11	18.3	-0.426
	-	-	-	OC4	12	124.0	0.664
PC4	14	93.4	0.162	OC5	14	28.2	0.392
PC5	15	53.9	0.364	OC6	15	16.0	-0.355

[1]Linkage group; [2]QTL position, distance from first marker in linkage group; [3]Additive main effects in percent protein/oil content