

QTL Mapping and Analysis of QTL x Nitrogen Interactions for Protein and Oil Contents in *Brassica napus* L.

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ABSTRACT

Nitrogen (N) is an important external input in plant production and has also significant impacts on product quality. To identify QTLs for oil and protein content and their interaction with N supply, 142 doubled haploid lines of a cross between 'Mansholts' and 'Samourai' were grown at four environments at two N levels: without N fertilisation and with a supply of 240 kg N/ha. For oil and protein content only small but statistically significant QTL x N interactions were observed. For oil content averaged over both N levels, in total 6 QTLs could be detected explaining about 78 % of the phenotypic variance. Four of these QTLs were at the same time QTLs for protein content, three of them influencing the two traits in opposite direction and explaining the negative correlation between oil and protein content in the seed.

Key words: rapeseed – seed quality - QTL - nitrogen – genotype x environment interaction

INTRODUCTION

The reduction of external inputs is of increasing importance in present agriculture. Nitrogen (N) fertilisers are among the most expensive inputs, and they also can cause environmental pollution by leaching to the ground water. Moreover, the nitrogen supply is of importance for the contents of oil and protein as major seed components in oilseed rape. The objectives were to identify quantitative trait loci (QTLs) for oil and protein content and their interaction with low and high levels of N supply, respectively.

MATERIAL AND METHODS

The QTL were mapped in a population of 142 segregating doubled haploid (DH) lines derived from a cross between the winter rapeseed cultivars 'Mansholt's Hamburger Raps' and 'Samourai'. Mansholt is an old cultivar with high erucic acid content and Samourai has canola quality. For QTL mapping, a framework map of 185 well spaced marker loci covering 1739 cM (Haldane) on 20 linkage groups was derived from an RFLP map (Uzunova et al., 1995).

In each of two years (1998/99 and 1999/2000), the DH lines were grown at two locations near Göttingen in two replications with two different N fertilizer treatments: unfertilized (N₀) and 240 kg/ha Nitrogen (N₁) total N supply. Three plants from each plot were harvested and analysed for oil and protein content by near infrared reflection spectroscopy (NIRS).

The software "QTLMapper" was used for the mixed model composite interval mapping (MCIM) and analysis of QTL interactions with N fertilization (Wang et al., 1999a, 1999b). First of all, QTLs were mapped separately for the two N levels. Then the two N levels were analysed together as two environments to estimate QTL x environment interactions. A likelihood-odds-ratio (LOD) score threshold of 1.71 was used at P≤0.005 for the detection of significant QTL effects. The QTL were first localized by scanning the linkage groups in 5 cM (Haldane) intervals. The QTL x N interactions were tested by a Jackknife procedure. The One-unit-down method was used to test whether QTLs at different environments or for different traits were located at statistically different positions (Lincoln et al., 1993).

RESULTS AND DISCUSSION

The parental line Mansholt had a higher oil and protein content than Samourai. Higher N fertilization resulted in both parents in a reduced oil content and an increased protein content

(Table 1). Among the DH lines, the variance due to genotype, N-level and genotype x N interaction was statistically significant (data not shown, see Gül, 2002).

Table 1. Mean values of the parents at different N levels ($N_0 = 0$ kg, $N_1 = 240$ kg/h)

Genotype	Oil content [%]		Protein content [%]	
	N_0	N_1	N_0	N_1
Mansholts (DH5.1/2)	48.67	46.31	16.98	20.22
Samourai (DH11.4)	47.22	44.59	16.11	19.33

In total 8 QTL for oil content were detected in at least one of the two N levels (Table 2). When mapping was based on the separate analyses of the two N levels, only two QTLs (OC2 and OC4) could be identified in both N levels. On two linkage groups (LG5 and LG11) QTLs were mapped in both N levels, however at statistically different positions. When analysing both N levels together, six QTLs were detected, explaining about 78 % of the phenotypic variance. Only one of these QTLs (OC8) showed a significant interaction with N supply, explaining less than 3 % of the phenotypic variance.

Table 2. QTL for oil content [%] at different N levels and their interaction with N supply (N_0 : 0 kg/ha; N_1 : 240 kg/ha)

N	QTL	LG ¹	Interval	P ²	A ³	EV ⁴	A (QTLxN) ⁵	EV (QTLxN) ⁶	LOD
N_0	OC1	5	MG18-MG19	0.0	0.53	7.8	-	-	5.43
	OC2	6	MG21-GATA.H3	16.0	1.17	37.5	-	-	21.45
	OC3	11	WG1F6.H1-OPB5.910	30.0	-0.44	5.2	-	-	2.93
	OC4	12	RP1218.H1-OPAG10.63	6.0	0.78	16.8	-	-	8.74
	OC5	15	RP1266.E1-RP436.E1	12.0	-0.43	5.1	-	-	2.91
						72.4			
N_1	OC6	5	RP1365.H4-RP1617.H1	0.0	0.39	6.1	-	-	3.34
	OC2	6	GATA.H3-OPS7.970	0.0	0.91	32.6	-	-	14.08
	OC7	11	WG6A11.H1-RP1068.E1	14.0	-0.59	13.9	-	-	5.82
	OC4	12	RP1218.H1-OPAG10.63	6.0	0.55	11.9	-	-	5.94
	OC8	14	OPAI16.14-RP1422.E1	2.0	0.44	7.8	-	-	3.69
						72.3			
N_0 & N_1	OC1	5	MG18-MG19	0.0	0.51	8.6	-0.03	Ns	6.51
	OC2	6	GATA.H3-OPS7.970	0.0	1.06	38.2	0.15	ns	34.17
	OC7	11	WG6A11.H1-RP1068.E1	12.0	-0.43	6.2	0.18	ns	6.29
	OC4	12	RP1218.H1-OPAG10.63	6.0	0.66	15.1	0.13	ns	14.37
	OC8	14	OPAI16.14-RP1422.E1	2.0	0.39	5.3	0.28	2.6	4.95
	OC5	15	RP1266.E1-RP436.E1	16.0	-0.34	4.3	0.01	ns	3.65
						77.7	2.6		

¹linkage group, ²distance [cM] for first marker of the interval, ³additive effect, substitution of one 'Samourai' allele by the allele from 'Mansholts', ⁴explained phenotypic variance by additive effect, ⁵effect of interaction between QTL and N level, ⁶explained phenotypic variance by QTL x N interaction, ns: effect not statistically significant

The two largest QTLs (OC2 on LG6 and OC4 on LG12) have been mapped before in an independent field experiment with the same population (Ecke et al., 1995). For both QTLs, the alleles for high oil content are from the Mansholts parent and are located in the same region as the two genes for erucic acid content. Probably the increased oil content in Mansholts is a pleiotropic effect of the alleles for high erucic acid due to the larger molecular weight of erucic acid compared to oleic acid (Ecke et al., 1995). In material of canola quality no segregation for these two QTLs can be expected, and the amount of variation in oil content explained by major QTLs might be smaller. For protein content, 5 QTLs were detected in the analysis of both N levels together, explaining about 33 % of the phenotypic variance (Gül 2002, data not shown). Several of these QTLs for protein content were mapped on the same linkage groups as QTLs for oil content (Table 3). The two major QTLs for oil content on LG6 and LG12 mentioned before do not affect protein content. The QTLs on linkage groups 5, 11, and 15 have opposite allelic effects on oil and protein content, which may explain the negative correlation ($r = -0.65^{**}$) that was observed in the 142 lines of the mapping population.

Table 3. Comparison of QTLs for oil and protein content; mean values over both N levels.

LG ¹	Oil content [%]			Protein content [%]		
	Interval	Add. effect ²	Position (cM)	Position (cM)	Interval	Add. effect ²
2	-	-	-	69.6	MG9-RP1087.H1	-0.22
5	MG18-MG19	0.51	16.1	17.9	MG19-RP1365.H4	-0.29
6	GATA.H3-OPS7.970	1.06	62.9	-	-	-
11	WG6A11.H1-	-0.43	26.3	57.3	WG1F6.H1-OPB5.910	0.23
12	RP1218.H1-OPAG10.63	0.66	129.3	-	-	-
14	OPI16.14-RP1422.E1	0.39	28.2	97.4	RP1117.E4-WG2D5.H1	0.16
15	RP1266.E1-RP436.E1	-0.36	16.1	53.9	RP150.E1-RP1362.E1	0.36

¹linkage group, ²additive effect, substitution of one 'Samurai' allele by the allele from 'Mansholts'

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REFERENCES

- Ecke, W., M. Uzunova and K. Weißleder, 1995: Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. *Theor. Appl. Genet.* 91, 972-977.
- Gül, M.K., 2002: QTL-Kartierung und Analyse von QTL × Stickstoff Interaktionen beim Winterraps (*Brassica napus* L.). Dissertation, Fachbereich Agrarwissenschaften der Georg-August-Universität, Göttingen. Cuvillier Verlag Göttingen, ISBN 3-89873-446-3
- Lincoln, S.E., M.J. Daly, and E.S. Lander, 1993: Mapping genes controlling quantitative traits using MAPMAKER/QTL version 1.1: A tutorial and reference manual. 2. ed., Whitehead Institute of Biomedical Research, Cambridge, USA.
- Uzunova, M., W. Ecke, K. Weißleder and G. Röbbelen, 1995: Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. *Theor. Appl. Genet.* 90, 194-204.
- Wang, D. L., J. Zhu, Z. K. Li and A. H. Paterson. 1999a. Mapping QTL with effects and QTL environment interactions by mixed linear model approaches. *Theor. Appl. Genet.* 99, pp. 1255-1264.
- Wang, D. L., J. Zhu, Z. K. Li and A. H. Paterson. 1999b. User Manual for QTLMapper Version 1.01b. A Computer Software for Mapping Quantitative Trait Loci (QTL) with Main Effects, Epistatic Effects and QTL x Environment Interactions. Department of Agronomy, Zhijiang University, Hagzhou 310029, China.