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QTL Mapping and Analysis of QTL x Nitrogen Interactions for Some Yield Components in *Brassica napus* L.

M. Kemal GÜL
Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crops, 17100, Çanakkale - TURKEY

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Abstract: Nitrogen (N) plays a critical role in crop production. Nowadays, it is possible to detect quantitative trait loci (QTL), as well as their effects and positions on chromosomes, by new molecular and biometrical methods. A doubled haploid rapeseed population of 142 lines from the doubled winter rapeseed cultivars Mansholt's and Samourai, and a framework map derived from an RFLP map were used for the analysis of QTL and their interactions with N in terms of some yield components obtained from field trials in *Brassica napus* L. The interactions between traits and N were determined by variance analysis. Two QTL for number of seeds per pod, and four QTL for thousand seed weight were mapped at N_0 (0 kg/ha N). Three QTL for pod length, two QTL for number of seeds per pod and three QTL for thousand seed weight were mapped at N_1 (240 kg/ha N). All the QTL of N_0 and N_1 were mapped by analysing QTL x N interactions for all traits. Although it was found that there was no interaction between the mapped QTL and N, the results here can be used to improve the N-use efficiency and seed yield of *Brassica napus*.

Key Words: *Brassica napus*, QTL (quantitative trait loci) mapping, nitrogen (N), interaction

Kolzada (*Brassica napus* L.) Verimle İlgili Bazı Karakterlere Ait QTL Haritalaması ve QTL ile Azot Arasındaki İnteraksiyon Analizleri

Özet: Azot bitkisel üretimde kullanılan en önemli çevre faktörlerinden biridir. Günümüzde kantitatif kalıtım gösteren karakterler ile ilgili genlerin yer aldığı lokusları (QTL= *kantitatif karakter lokusları*) ve bu segmentlerin kromozom üzerindeki yerleri yeni moleküler ve biyometriksel yöntemlerle saptamak mümkündür. Yapılan bu çalışmada, iki farklı kolza çeşidi olan Mansholt's ve Samourai'in katlanmış haploid bitkileri birbiriyle melezlenip bu melezlerin mikrosporları yoluyla elde edilen katlanmış haploidlerden (DH: doupled haploid) oluşan bir populasyon ile RFLP (restriction fragment length polymorphism) markörlerinden oluşturulmuş bir harita kullanılmıştır. Verimle ilgili bazı karakterler için QTL analizleri yapılmış ve bulunan QTL'lerin azot (N) ile olan interaksiyonlar varyans analizleri ile incelenmiştir. Gübrelenmemiş (N= 0 kg/ha) denemede kapsüldeki tohum sayısı için iki, bin dane ağırlığı için de dört QTL haritalanmıştır. Gübrelenmiş (N= 240 kg/ha) denemede ise kapsül uzunluğu için üç, kapsüldeki tohum sayısı için iki ve bin tohum ağırlığı için de üç QTL bulunmuştur. Gübresiz ve gübreli denemelerde incelenen karakterler için bulunan bütün QTL, QTL x azot interaksiyonları analizleri sırasında da teyit edilmiştir. Elde edilen sonuçlar QTL`ler ve N arasında herhangi bir interaksiyon ortaya koymamakla birlikte kolzada azot kullanım etkinliğini artırma ve verimi iyileştirilme çalışmalarında faydalı olabilir.

Anahtar Sözcükler: *Brassica napus* L., QTL (kantitatif karakter lokusları) haritalaması, Azot (N), interaksiyon

Introduction

Brassica napus is an important oil-seed crop, the second most widely grown after soyabean, with an annual production of 38 million tonnes worldwide (Fried et al., 2002).

Generally, the first aim of a plant breeder is to improve yield. Nitrogen (N) is a major input in plant production. Reducing N application without decreasing yield white improving N-use efficiency would be an important aim.

All of the traits responsible for yield are inherited quantitatively (polygenic). Hence it is not always possible to observe any distinct segregation in F2 or following generations after crossing in terms of grain yield. Furthermore, all quantitative traits are probably influenced by the environment. The effects of genes for

Correspondence to: mkemalgul@yahoo.de

quantitative traits and their interactions with environment have been intensively studied with the help of new molecular and biometrical methods since the last decade (Lander and Botstein, 1989; Utz and Melchinger, 1996; Wang et al., 1999).

The aim of this study was to map the quantative trait loci (QTL) and analyse the interactions between QTL and N application for some yield traits by the use of additive main effects.

Materials and Methods

The QTL were mapped in a segregating double haploid (DH) population originally derived from microspores of F1 plants of a cross between doubled lines of the winter rapeseed cultivars 'Mansholt's Hamburger Raps' (DH5.1/2) and 'Samourai' (DH11.4). Mansholt's Hamburger Raps is an old land race with a high erusic acid content, and Samourai is a new variety with canola quality. For the production of DH lines microspores of oilseed rape (n = 19) were grown in a suitable medium in order to obtain haploid plantlets. These were then developed in cholchicin to double the chromosome numbers. Thus, doubled plants which were 100% homozygous were obtained in only one step. The most important features of DH are to set up an experiment using such populations and to enable researchers to obtain reliable mapping analyses since no segregation in the lines will occur.

For QTL mapping, a framework map of 185 well-spaced marker loci covering 1739 cM (Haldane) on 20 linkage groups (LG) was derived from an restriction fragment length polymorphism (RFLP) map, developed in the DH population at the Institute of Agronomy and Plant Breeding in Göttingen, Germany (Uzunova, 1994). LG were made according to the chromosome number of oilseed rape (n = 19) in a program called MAPMAKER (Lander et al., 1987). Those markers that would not fit in are grouped in LG 20.

For two consecutive years, namely the 1998/99 and 1999/2000 growing seasons, 142 lines of DH population and their parents were grown at two locations in double rows (2.5 m, 80 plants per double row) in Göttingen in two replications with two different N fertiliser treatments: control (N_0) and 240 kg/ha nitrogen (N_1).

The investigated traits for yield were the pod length (PL), number of seeds per pod (NSP) and thousand seed weight (TSW). Three pods from the main shoots and three plants from each plot were harvested for the evaluation. The estimation of variance components was carnied out using the program PLABSTAT (Utz, 1997). Mapping analyses were performed using a computer program according to the data obtained from field experiments along with the map of a population. The mapping program for this study, QTLMapper, was used for the mixed-model composite interval mapping (MCIM) and analysis of QTL interactions with N fertilisation (Wang et al., 1999). Initially, for each DH line, trait means for each N level were calculated separately from both years and locations, and were used for QTL mapping. The mean values of both N levels were used for the analysis of QTL x N interactions. Therefore, the N fertiliser treatments were entered as two different environments during the analyses.

A likelihood-odds- ratio (LOD) score threshold of 1.71 was used at $P \leq 0.005$ for the detection of significant QTL effects. The QTL were first localised by scanning the linkage groups in 5 cM (Haldane) intervals. The MCIM for putative QTL were mapped by the QTL mapping command. The most likely position of QTL was calculated by the Filtration command. Finally, the QTL x N interactions were analysed by the integrated Jackknife test.

Results

The variance analysis showed no significant differences between parents and mean values of DH lines. However, significant variations were found within DH lines (Table 1). Tables 2, 3 and 4 present the significant mapped QTL ($1.7 \leq$ LOD). The LOD values were not lower than 2.50 for any traits.

No QTL were mapped for PL at N_0. However, three QTL were mapped at the N_1 level. The cumulative main effects of QTL explained 27% of the phenotypic variance. The same QTL were mapped for the analyses of QTL x N interaction. All the QTL explained 21% of the fenotypic variance. No interaction was found between QTL and N fertilisation, possibly due to the small interaction between the genotype and the N application (Table 2).

Two QTL were mapped for NSP at both N levels and these explained 20% of the phenotypic variance. At NO and N_1, NSP1 was mapped at the same position on linkage group 15. NSP2 was mapped on LG 18 only at N_0.

Table 1. The minimum, maximum and the mean values of the parents and DH lines at different N levels (N_0 = 0 kg, N_1 = 240 kg/h).

Genotype	Pod length (PL) (cm)		Number of seeds per pod (NSP)		Thousand seed weight (TSW) (g)	
	N_0	N_1	N_0	N_1	N_0	N_1
DH5.1/2	6.41	6.11	18.07	15.62	4.15	4.83
DH11.4	6.13	6.13	17.67	15.4	4.45	5.04
Mean value	6.27	6.12	17.87	15.51	4.3	4.93
DH lines						
Mean	6.36	6.07	17.91	16.37	4.11	4.61
Minimum	3.56	2.83	5.22	2.78	2.82	2.57
Maximum	10.72	15.83	32.33	32.56	5.99	6.37
SD	0.84	1.12	5.03	5.65	0.53	0.69
$LSD_{0.05}$	0.59	0.73	3.13	3.61	0.34	0.40

SD: Standard deviation, LSD: Least significant difference

Table 2. The mapped QTL for PL at different N levels and their position on linkage groups.

N	QTL[1]	LG[2]	Interval[3]	A[4]	EV[5] (%)	A (QTL x N)[6]	EV (QTL x N)[7]	LOD[8]
N_1	PL1	4	RP438.E1-RP1042.H1	0.145	8.76	-	-	5.25
	PL2	8	MG25-MG26	-0.169	11.90	-	-	6.26
	PL3	15	RP1413.H2-cRT68.H1	0.124	6.40	-	-	3.19
	Heritability (broad sense): 0.73				27.06			
	Variance							
	genotypic: 0.18, phenotypic: 0.24							
	PL1	4	RP438.E1-RP1042.H1	0.096	5.12	-0.051	0.00	3.45
	PL2	8	MG25-MG26	-0.129	9.24	0.044	0.00	6.40
N_0	PL3	15	RP1413.H2-cRT68.H1	0.116	7.47	-0.009	0.00	4.59
&	Heritability (broad sense): 0.80				21.83		0.00	
N_1	Variance							
	genotypic: 0.14, phenotypic: 0.18							
	G x N- interaction: 0.01* significant at 0.05							

1: QTL name, 2: Linkage group, 3: Marker interval on LG, 4: Additive main effect, 5: Explained phenotypic variance by additive main effect, 6: Contribution of N to additive main effect, 7: Explained phenotypic variance by QTL x N interaction, 8: LOD: Likelihood-odds ratio (LOD > 1.71).

On LG 18, NSP3 was mapped in another marker interval at N_1 (Table 3). All QTL were mapped at the same intervals for the QTL x N interaction analysis. This trait showed significant interaction with N fertilisation. However, no interaction between QTL and N fertilisation was found.

Four QTL were mapped for TSW on three different LGs at the N_0 level. Two QTL were identified in two independent intervals on the LG 3. The third of the QTL was found on LG 5, and the last one was mapped on LG 12. All the QTL explained 49% of the phenotypic variance (Table 4). The QTL mapped at the N_0 level were identified in the same position at the N_1 level, except for TSW4 on LG 12. They explained 39% of the phenotypic variance. The QTL on LGs 3, 5 and 12 were also mapped for the analysis of QTL x N interaction in the same position. TSW showed no significant interaction between genotype and N fertilisation. All QTL for TSW were found by analysing the QTL x N interactions, and these explained 43% of the phenotypic variance.

PL and NSP were positively correlated (r_1 = 0.51** at N_0, r_2 = 0.64** at N_1, $r_{N0\&N1}$ = 0.58**) although the

Table 3. The mapped QTL for NSP at different N levels and their position on linkage groups.

N	QTL[1]	LG[2]	Interval[3]	A[4]	EV[5] (%)	A (QTL x N)[6]	EV (QTL x N)[7]	LOD[8]
N_0	NSP1	15	RP1435.E1-RP150.E1	-0.591	9.59	-	-	3.99
	NSP2	18	RP1365.H1-OPD20.840	0.608	10.15	-	-	4.11
	Heritability (broad sense): 0.65				19.74			
	Variance							
	genotypic: 2.368, phenotypic: 3.64							
	NSP1	15	RP1435.E1-RP150.E1	-0.536	8.65	-	-	2.96
	NSP3	18	WG7A8.H1-WG4E12.H1	0.615	11.39	-	-	3.92
N_1	Heritability (broad sense): 0.50				20.14			
	Variance							
	genotypic: 1.66, phenotypic: 3.32							
	NSP1	15	RP1435.E1-RP150.E1	-0.563	11.69	-0.030	0.00	6.55
	NSP2	18	RP1365.H1-OPD20.840	0.385	5.47	0.230	0.00	3.85
N_0	NSP3	18	WG7A8.H1-WG4E12.H1	0.370	5.05	-0.248	0.00	3.09
&	Heritability (broad sense): 0.67				22.21		0.00	
N_1	Variance							
	genotypic: 1.82, phenotypic: 2.71							
	G x N- interaction: 0.38* significant at 0.05							

1: QTL name, 2: Linkage group, 3: Marker interval on LG, 4: Additive main effect, 5: Explained phenotypic variance by additive main effect, 6: Contribution of N to additive main effect, 7: Explained phenotypic variance by QTL x N interaction, 8: LOD: Likelihood-odds ratio (LOD > 1.71).

Table 4. The mapped QTL for TSW at different N levels and their position on linkage groups.

N	QTL[1]	LG[2]	Interval[3]	A[4]	EV[5] (%)	A (QTL x N)[6]	EV (QTL x N)[7]	LOD[8]
N_0	TSW1	3	RP830.E1-RP1122.H1	0.106	12.48	-	-	6.11
	TSW 2	3	RP1466.H1-RP1214.E1	0.111	13.69	-	-	7.33
	TSW 3	5	RP1275.H2-RP1165.H1	0.123	16.81	-	-	8.20
	TSW 4	12	RP1471.H1-WG5B1.H1	0.075	6.25	-	-	4.20
	Heritability (broad sense): 0.85				49.23			
	Variance							
	genotypic: 0.08, phenotypic: 0.09							
N_1	TSW 1	3	RP1146.H3-RP830.E1	0.084	5.88	-	-	2.50
	TSW 2	3	RP1466.H1-RP1214.E1	0.174	25.23	-	-	9.63
	TSW 3	5	RP1501.H1-RP1275.H2	0.097	7.84	-	-	3.26
	Heritability (broad sense): 0.83				38.95			
	Variance							
	genotypic: 0.1, phenotypic: 0.12							
	TSW 1	3	RP1146.H3-RP830.E1	0.089	7.92	0.009	0.00	6.56
	TSW 2	3	RP1466.H1-RP1214.E1	0.141	19.88	-0.034	0.00	16.43
N_0	TSW 3	5	RP1501.H1-RP1275.H2	0.108	11.66	0.013	0.00	9.43
&	TSW 4	12	RP1471.H1-WG5B1.H1	0.059	3.48	0.020	0.00	3.62
N_1	Heritability (broad sense): 0.90				42.92			
	Variance							
	genotypic: 0.09, phenotypic: 0.10							
	G x N- interaction: 0.00 not significant						0.00	

1: QTL name, 2: Linkage group, 3: Marker interval on LG, 4: Additive main effect, 5: Explained phenotypic variance by additive main effect, 6: Contribution of N to additive main effect, 7: Explained phenotypic variance by QTL x N interaction, 8: LOD: Likelihood-odds ratio (LOD > 1.71).

positions and effects of QTL for PL and NSP showed no similarities. However, two QTL for each trait were indentified on LG 18 at different intervals, which contributed to higher PL and NSP values.

TSW showed no correlation with any trait. This can be explained by the absence of pleiotropic gene effects for the investigated traits.

Discussion

QTL were mapped for all traits under different conditions. In this DH population analysed for all three traits, small genotype interactions with N fertilisation were not compared to genetic effects. However, using a QTL mapping approach with integrated analysis of QTL x environment, interaction can be assigned to individual QTL. If the phenotypic variance explained by a single QTL is high, then it is possible to calculate significant interactions between a single QTL and environmental factors. Due to the small effects of a large number of QTL or genes for a trait, it becomes difficult to calculate significant interactions for each single QTL and environmental factors, although there is a significant interaction found between genotype and environment by variance analysis. Gül (2002) found QTL x N interactions in the same population for protein and oil content showing high heritability. QTL x N interaction of one QTL for protein content explained a maximum of 6% of phenotypic variance, and because of the significant interaction between protein and N fertilisation that was the highest value in this study.

The use of QTL x environment interactions is important for the breeding of so-called "special varieties" which show a high interaction with the environment. In this study, no significant interactions between the three traits and N fertiliser were found. In general, an interaction between genotype and the environment is not desired for yield safety in plant breeding (Becker, 1993). The identification of QTL for PL can be explained by special genes, which were mapped only at the N_1 level.

It is expected that the same genome segment or segments would be mapped for high correlated traits. PL and NSP were highly correlated. However, no QTL were mapped for both traits, and this can be explained by the fact that the genes for both traits are independent. If QTL were detected common to both traits, then this could be explained by the pleitropic effects of genes or linked genes (Falconer, 1984). This has been confirmed in earlier studies using different plants (Wissuwa and Ae, 1999; Kicherer et al., 2000).

The QTL TSW2 explained a maximum of 25% of the phenotypic variance at the N_1 level. The same QTL explained 14% of the phenotypic variance at the N_0 level, both exhibiting high values for one QTL. The characterisation and selection for these QTL are simple, providing easy selection for high TSW under different N fertilisation. The genes for quantitative traits with high additive effects are referred to as "major genes" with a high contribution to the explained variance. In similar studies major genes were mapped for glucosinolate content in *Brassica napus*. Three QTL alone explained as much as 89% of the phenotypic variance for glucosinolate content (Weissleder, 1996; Gül, 2002).

In principle, QTL can be used for MAS (marker assisted selection). With the help of markers it is possible to begin with specific selection in earlier generations. There are some conditions for an indirect and successful selection (Haensel, 1976; Falconer and Mackay, 1996): easy evaluation of traits, high heritability and high correlation between complementary traits and target traits. In addition, the number of individuals of a population, the number of trials, the number of markers and the mapping program used are also important. With the DH population, the same trials can be repeated in different years. The linkage map must be covered with as many markers as possible. There are several programs that give different results from the same data, because of different preferences of co-factors and LOD; this is very important for traits with low heritability in particular. Under these conditions, QTL mapping can be done safely.

The results showed that the investigated traits were not influenced by N fertilisation. To optimise N fertilisation and improve N-use efficiency, the yield components and the yield itself can be successfully used for indirect selection. However, it should still be kept in mind that yield is a complex trait and many factors, such as resistance to a broad spectrum of biotical and abiotic factors, could contribute to this trait (Presterl et al., 2000). Molecular markers and QTL mapping will be widely used in the breeding approaches of the future.

References

Becker, H.C. 1993: Pflanzenzüchtung. Ulmer Verlag, Stuttgart.

Gül, M.K. 2002. QTL-Kartierung und Analyse von QTL x Stickstoff Interaktionen beim Winterraps (*Brassica napus* L.). Ph.D. thesis. Universitaet Göttingen, Germany, pp. 34-38.

Falconer, D.S. 1984: Einführung in die quantitative Genetik. Eugen Ulmer Verlag, Stuttgart.

Falconer, D.S. and T.F.C. Mackay. 1996: Introduction to Quantitative Genetics. 4th Edition. Longman Scientific Technical, New York.

Fried, W., R. Baetzel, A.G. Badani, M. Koch, R. Schmidt, R. Horn and W. Lühs. 2002. Züchtung auf optimierte Schrotqualitaet bei Raps (*Brassica napus*). Vortr. Pflanzenzüchtung. 54: pp. 131-143.

Haensel, H. 1976. Heritabilitaet von Teileigenschaften und deren Korrelation zu Komplexeigenschaften ("Tafeln für indirekte Selektion"). Ber. Arb. Tag. Gem. Saatzuchtleiter, Gumpenstein, pp. 3-10.

Kicherer, S., G. Backes, U. Walther and A. Jahoor. 2000. Localising QTL for leaf rust resistence and agronomic traits in barleys (*Hordeum vulgare* L.). Theor. Appl. Genet. 100: pp. 881-888.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1: pp. 174-181.

Lander, E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: pp. 185-199.

Presterl, T., E. Thiemt and H.H. Geiger. 2000. Züchtung von Mais mit verbesserter Stickstoffeffizienz. In: Möllers, C. (Hrsg.) Stickstoffeffizienz Landwirtschaftlicher Kulturpflanzen. Erich Schmidt Verlag, Berlin, pp. 148-153.

Utz, H.F. and E. Melchinger. 1996: PLABQTL: A program for composite interval mapping of QTLs. (http://www.uni-hohenheim.de/i3v/00068900/18314041.htm 12.09.2000).

Utz, H.F. 1997: Plabstat: Ein Computerprogramm zur statistischen Analyse von pflanzenzüchteriscen Experimenten. Version 2N. Institut für Pflanzenbau und Pflanzenzüctung, Universitaet Hohenheim (http://www.uni-hohenheim.de).

Uzunova, M. 1994. Erstellung einer RFLP-Karte von Raps (*Brassica napus*) und Kartierung erster züchterisch wichtiger Gene. Ph.D. thesis. Universitaet Göttingen, pp. 23-53.

Wang, D.L., J. Zhu, Z.K. Li and A.H. Paterson. 1999. Mapping QTL with effects and QTL environment interactions by mixed linear model approaches. Theor. Appl. Genet. 99: pp. 1255-1264.

Weissleder, K. 1996. Genetische Kartierung von Loci für züchterisch bedeutsame Merkmale beim Winteraps (*Brassica napus* L.). Ph.D. thesis. Universitaet Göttingen, pp. 75-78.

Wissuwa, M. and N. Ae. 1999. Molecular markers associated with phosphorus uptake and internal phosphorus-use efficiency in rice. In: Plant Nutrition-Molecular Biology and Genetics Eds.: Gissel-Nielsen and A. Jensen, Kluwer Academic Publishers. Printed in the Netherlands, pp. 433-439.