

QTL effects for seed glucosinolate content in BC₃ families of rapeseed (*Brassica napus* L.) segregating for single QTL only

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ABSTRACT

Six QTL for glucosinolate content have been mapped in previous studies in a segregating doubled haploid population derived from a cross between the old cultivar 'Mansholt' and the canola quality winter rapeseed variety 'Samourai'. Three of these QTL have been reported to have major effects while the remaining three showed only minor effects. In the present study a BC₂ population derived from the same cross with 'Samourai' as recurrent parent was genetically characterized using 143 molecular markers. Based on the marker information BC₃ families segregating for only one of the three major QTL were selected. The BC₃ plants were grown in field trials and after harvest seed glucosinolate content was evaluated using NIRS. The objective of this study was to estimate the effects of the 3 major QTLs for glucosinolate content. The BC₃ families are part of a program which aims at the development of a set of intervarietal substitution lines.

Key words: Glucosinolates, QTL, AFLP, NIRS, marker.

INTRODUCTION

After extraction of the seed oil, the main product of rapeseed cultivation, the remaining meal is used as an important protein source in animal feeding. Since glucosinolates are detrimental to animal nutrition it still remains a breeding objective to have varieties with low glucosinolate content. Glucosinolates have different biological effects, ranging from antimicrobial and cancer preventing to goitrogenic activities (Wittstock and Halkier, 2002). Six QTLs for glucosinolate content have been identified until now in a segregating doubled haploid population derived from a cross between the old cultivar 'Mansholt' (high content of both erucic acid and glucosinolates) and the canola quality winter rapeseed variety 'Samourai'. Three of these QTLs on linkage groups 9, 16, and 18, have been reported to have major effects while the other three on linkage groups 2, 3, and 6 showed only minor effects (Uzunova et al. 1995, Weißleder 1996, Fischer and Ecke 1997, Gül 2002).

MATERIALS AND METHODS

The genetic map of rapeseed developed by Uzunova *et al.* (1995) was the starting point for this research. Ten selected DH lines with donor segments covering all the Mansholt's genome were backcrossed twice to DH 11.4 (Samourai) to produce 300 BC₂ plants. Plants were grown in the greenhouse and leaf samples were taken for DNA isolation. The plants were backcrossed to generate BC₃ seeds. AFLP reactions were performed according to Vos et al. (1995). 13 AFLP primer combination, two SSR primers and one RAPD primer were used to analyse the 300 BC₂ plants. Fragment analysis of AFLP amplification products was performed on a sequencer (4200 Li-Cor® DNA Analyser). Digital gel images were scored visually or using the software AFLP-Quantar™ Pro 1.0 (KeyGene Products B.V.). SSR and RAPD markers were analysed as described by Uzunova and Ecke (1999) and Uzunova *et al.* (1995), respectively.

279 BC₃ families, the 10 selected DH lines and their derived BC₁ genotypes as well as the parental lines DH 11.4 (Samourai) and DH 5.1 (Mansholt), were grown in 2001/2002 in field trials at two locations in plots with 50 plants. Seed glucosinolate content was determined in intact-seed samples (300 mg) by near infrared reflectance spectroscopy (NIRS) using the instrument FOSS NIRS Systems/TECATOR (Model 6500) and the software ISI version 1.04 (Infrasoft International, LLC.).

QTL effects in BC₃ families segregating for only one of the QTL were calculated as the difference between phenotypic classes. In families where phenotypic classes could not be clearly

distinguished the QTL effect was calculated by comparing the phenotypic mean (z) of the BC_3 family with the phenotypic mean (y) of the recurrent parent Samourai under the assumption that “ z ” can be calculated as “ $z = (x + y)/2$ ” with “ x ” being the phenotypic mean of the heterozygous class and “ y ” of the homozygous class, which corresponds to Samourai. In this way, in BC_3 families segregating for only one QTL the phenotypic mean of the heterozygous class can be calculated as “ $x = (2z) - y$ ”, and the QTL effect as “ $x - y$ ”.

RESULTS

The BC_2 population of 300 plants was characterized using 140 AFLP, 2 SSR and 1 RAPD marker covering about 1325 cM of the rapeseed genome. Based on the marker data, BC_3 families were selected which segregated for only one of the 3 major QTL and none of the minor QTL, and seed glucosinolate content was determined individually from all plants of these families. For the two major QTL on linkage groups 16 (Fig. 1) and 18, clear 1 : 1 segregations into two phenotypic classes were observed. For the major QTL on linkage group 9 no clear phenotypic segregation could be observed. Table 1 shows a comparison of the QTL effects as determined in the BC_3 families with the additive effects estimated by interval mapping in different experiments using the segregating double haploid populations from the cross ‘Mansholt’ x ‘Samourai’.

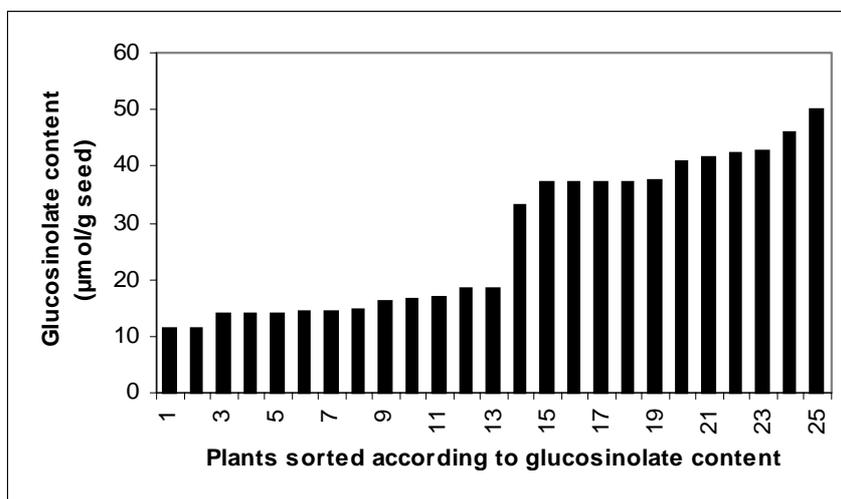


Fig. 1. Distribution of glucosinolate content in BC_3 family 2034-2-18 (in one location) segregating for the QTL on linkage group 16.

DISCUSSION

In the present study the segregation and the phenotypic effects of the major QTL for glucosinolate content have been analysed in BC_3 families, each segregating for only one of the QTL. For two QTL a clear segregation into two phenotypic classes could be observed, indicating that it should be possible to map these QTL as specific genes. This would allow a precise localization of the QTL on the genetic map of rapeseed and possibly the cloning of these QTL, either by a map-based approach or by utilizing the synteny between rapeseed and *Arabidopsis*.

In the BC_3 families the effects determined for the QTL are the sum of the additive and dominance effects. A comparison with the additive effects estimated by interval mapping indicates the presence of strong dominance effects. For the QTL on linkage group 18 additive and dominance effects are nearly equal. For the other two QTL the dominance effects are larger than the additive effects. On the other hand, the sum of the QTL effects estimated in the BC_3 families exceeds the difference in glucosinolate content between the parents by 9.5 µmol/g. Furthermore, when analysing the parental lines and the F_1 of the cross ‘Mansholt’ x ‘Samourai’ Uzunova et al. (1995) and Weißleder (1996) observed only partial dominance for glucosinolate

content. This may indicate epistatic interactions that reduce phenotypic effects of 'Mansholt', alleles when these alleles are present at more than one of the QTL. Such interaction effects may also have led to an underestimation of additive effects by interval mapping since many of the doubled haploid lines of the mapping population have 'Mansholt' alleles at more than one of the QTL and interaction effects were not included in the model used for QTL mapping.

Table 1 QTL effects for glucosinolate content [$\mu\text{mol/g}$ seed] estimated in different studies in the cross 'Mansholt' x 'Samourai' and phenotypic values of the parents.

Linkage Group (LG)	QTL effects estimated by interval mapping ¹					Effects in BC ₃ fam. ² 2002	No. of families analysed
	Uzunova <i>et al.</i> ³	Weileder ⁴	Fisher and Ecke ⁵	Gl ⁶	Mean		
LG9	4.3	4.6	5.4	6.8	5.3	18.6	4
LG16	7.8	8.3	8.3	10.4	8.7	22.9	6
LG18	3.5	5.9	6.5	6.1	5.5	12.0	4
Mansholt ⁷	62.3	52.4	51.9	65.2	57.9	60.9	
Samourai ⁷	11.2	13.7	13.5	16.5	13.7	16.8	

¹ Additive effect due to the substitution of a 'Samourai' allele by a 'Mansholt' allele.

² Additive+dominance effects in BC₃ families

³ 1995, ⁴ 1996, ⁵ 1997, ⁶ 2002

⁷ Phenotypic values

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