QTL analysis of phytosterol content in rapeseed (*Brassica napus* L.)

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Abstract

Phytosterols are natural plant oil constituents. In rapeseed, one of the richest natural sources of phytosterols, four major phytosterols were identified: sitosterol, campesterol, *brassica*sterol and avenasterol. A further increase of phytosterol content could give a higher added value to rapeseed oil and oil-derived products. The objective of this study was to identify QTL for individual and total phytosterol content in a winter rapeseed doubled haploid (DH) population. The DH population showed highly significant genetic differences in individual and total phytosterol content. Three QTL controlling total phytosterol content were mapped on linkage groups 6, 12 and 20, explaining 60% of the total genetic variance. The QTL on linkage groups 6 and 12 showed the highest additive effects and were located at the similar position as the QTL detected for all individual phytosterols, indicating the presence of one or more genes at these positions affecting the content of all phytosterols. However, the QTL on the linkage groups 6 and 12 mapped within the confidence intervals of the two erucic acid genes. This suggests that the two erucic acid genes exert a pleiotropic effect on phytosterol content, which is in accordance with the observed close negative correlation between erucic acid and phytosterol content. The observed effect may be explained by the competitive use of cytoplasmic acetyl-CoA, the common precursor of both biosynthetic pathways.

Key words: phytosterols, QTL mapping, erucic acid

Introduction

Phytosterols are known since more than 50 years for their LDL-cholesterol-lowering properties. In rapeseed, one of the richest natural sources of phytosterols (Gordon and Miller 1997), four major phytosterols were identified: sitosterol, campesterol, *brassica*sterol and avenasterol. Nevertheless, a further increase of the phytosterol content could give an added value to rapeseed oil and oil-derived products. QTL responsible for different seed quality traits such as linolenic and erucic acid, oil, glucosinolates and tocopherols have been identified earlier on molecular linkage maps of the rapeseed genome (Marwede *et al*. 2005, and references therein). However, so far no QTL have been mapped for phytosterol content.

In a separate study, the genetic variation for phytosterol content and composition was analysed in three different doubled haploid (DH) populations (Amar *et al*. 2006). In this study, a DH population derived from a cross between the old Dutch cultivar 'Mansholt's Hamburger Raps' and the modern French cultivar 'Samourai' showed the largest segregation for individual and total phytosterol content. The aim of the present study was to map QTL for individual and total phytosterol content in this population.

Materials and Methods

Plant material and molecular marker map: The mapping population consisted of 148 DH lines derived from a cross between two DH lines obtained from the old Dutch cultivar 'Mansholt's Hamburger Raps' (high contents of erucic acid and glucosinolates) and from the modern French cultivar 'Samourai' (low contents of erucic acid and glucosinolate). The DH lines were grown in a field trial during two consecutive years at two locations in a randomised block design with two replications. Each plot consisted of a double row with around 80 plants/plot. In 1999, the two locations were two fields at Reinshof (4 km SW of Göttingen, Germany) with different soil types. In 2000, one location was Reinshof and the other was Weende (5 km NW of Göttingen). Seeds harvested from three open pollinated plants were bulked for the analysis (Gül 2002).

The QTL were mapped on a framework map comprising 185 evenly distributed markers selected from a previously established primary RFLP and AFLP map (unpublished data). The primary map comprised 482 markers distributed on 20 linkage groups and covered 1745 cM (Kosambi 1944) of the rapeseed genome. Means for phytosterol phenotypic data over all environments were used for composite interval mapping performed with the program PLABQTL (Utz 2006). Putative QTL were detected by using a LOD score threshold of 2.82 corresponding to a 5% probability of falsely declaring a QTL anywhere in the genome.

Analysis of phytosterol content and other quality traits: A gas-liquid chromatographic method was developed and used for the analysis of phytosterol content in seeds (Amar *et al*. 2006). Near-infrared reflectance spectroscopy (NIRS) was applied to determine the oil, erucic acid and sinapate ester content of the seed samples.

Results and Discussion

In the DH population four individual phytosterols were identified: sitosterol, campesterol, *brassica*sterol and avenasterol (Table 1). Sitosterol was the most prominent phytosterol, accounting for 53% of the total phytosterol content, followed by campesterol (29%), *brassica*sterol (14%) and avenasterol (4%). Total phytosterol content ranged from 2570 to 4104 mg/kg seed. The largest ranges of individual phytosterols were ascertained for the two most prominent phytosterols, sitosterol (1251-

2138 mg/kg seed) and campesterol (631-1533 mg/kg seed). The analysis of variance for all phytosterol traits revealed highly significant genetic effects (data not shown) with high heritabilities (Table 1).

Table 1. Genetic variation of phytosterol content (mg/kg seed) in the DH mapping population.

	*Brassica*sterol	Campesterol	Sitosterol	Avenasterol	Total
Mean	415	903	1648	129	3107
Min	333	631	1251	61	2570
Max	548	1533	2138	303	4104
F-value[§]	14**	14**	10**	7**	11**
LSD 0.05	31	108	147	51	262
h^2	0.93	0.93	0.90	0.86	0.91

[§] F-test from analysis of variance for genetic variation among DH lines
** significant at p = 0.01

Table 2. Genetic correlation between different seed quality traits in the DH mapping population.

	Brassica-sterol	Campe-sterol	Sito-sterol	Avena-sterol	Total phytosterols	Oil	Erucic acid
Campesterol	0.38^{++}						
Sitosterol	0.16^{+}	0.58^{++}					
Avenasterol	0.07	0.38^{++}	0.29^{++}				
Total phytosterols	0.40^{++}	0.88^{++}	0.87^{++}	0.50^{++}			
Oil	-0.32^{++}	-0.50^{++}	-0.64^{++}	-0.39^{++}	-0.67^{++}		
Erucic acid	-0.39^{++}	-0.76^{++}	-0.80^{++}	-0.49^{++}	-0.91^{++}	0.74^{++}	
Sinapate esters	0.26^{++}	0.48^{++}	0.67^{++}	0.34^{++}	0.67^{++}	-0.81^{++}	-0.77^{++}

[+] coefficient is larger than the standard error
[++] coefficient is two times larger than the standard error

Table 3. Mapped QTL and their most likely positions for total and individual phytosterol content.

Trait	LG[a]	D[b]	Marker interval	LOD score	a[c]	Vp%[d]	Vg%[d]	Total Vp%[e]	Total Vg%[e]
Total phyto-sterols	6	62	MG21-GATA.H3	5.9	205	47	52	54	60
	12	136	OPAG10.630-RP318a.E1	22.9	172	34	37		
	20	0	OPAG4.620-MG87	4.7	61	8	8		
Sito-sterol	6	58	MG21-GATA.H3	32.2	91	39	43	61	68
	8	26	RP1470.H1-WG7B3.H1	4.0	22	4	4		
	11	8	RP1454.E1-WG7E10.H1	16.7	-70	30	33		
	12	134	OPAG10.630-RP318a.E1	30.2	76	29	33		
Campe-sterol	6	60	MG21-GATA.H3	21.1	74	31	34	44	48
	12	24	WG9A2.E1-RP1117a.E2	10.6	28	6	7		
	12	146	RP318a.E1-RP1365.H3	9.2	54	19	21		
	14	76	RP1142.H1-RP1117a.E4	11.8	31	8	8		
	17	42	RP1230.H1-RP1117a.E1	7.6	44	14	15		
Brassica-sterol	6	64	GATA.H3-OPS7.970	8.5	12	13	14	48	52
	8	8	MG25-MG26	10.2	-10	10	11		
	9	56	RP1100.E1-RP825.H1	10.7	9	7	7		
	10	22	RP984.H2-MG40	5.8	8	7	8		
	12	128	RP1218.H1-OPAG10.630	6.1	11	11	12		
	14	28	OPAI16.1420-RP1422.E1	10.6	8	7	8		
	17	44	RP117a.E1-WG4A4.H1	4.9	-22	33	36		
	19	48	OPT9.862-RP981.H1	3.7	8	7	7		
Avena-sterol	5	40	GATA.H1-RP1126.H1	9.5	22	27	32	50	58
	6	36	OPA15.896-MG21	4.1	15	6	19		
	7	10	RP1202.H1-RP318b.E1	5.7	-7	4	4		
	12	144	RP318a.E1-RP1365.H3	3.0	12	10	12		
	13	12	OPA18.600-cRT21.E1	3.0	-16	13	15		
	14	76	RP1142.H1-RP1117a.E4	2.7	-20	25	29		

[a] linkage group
[b] distance from the first marker of the linkage group in cM
[c] additive effect (mg/kg seed) estimated for the substitution of a 'Mansholts' allele by a 'Samourai' allele
[d] proportion of phenotypic (Vp) or genetic (Vg) variance explained by the additive effect of the QTL
[e] proportion of total phenotypic (Vp) or total genetic (Vg) variance explained by the QTL, adjusted (Utz *et al*. 2000 and 2006)

Coefficients of genetic correlations between different quality traits are presented in Table 2. Positive correlations between total phytosterol and all individual phytosterols were observed. Individual phytosterols were positively correlated with each

other. A close negative correlation was detected between total phytosterols, oil content and erucic acid, respectively. Oil content was positively correlated to erucic acid content, which has been described and explained earlier (Ecke *et al*. 1995). Interestingly, a positive correlation was also found for phytosterols and sinapate esters.

Three QTL were identified for total phytosterol content on linkage groups (LG) 6, 12 and 20 (Table 3). The QTL on LG 6 showed the highest additive effect, explaining 52% of the genetic variance, followed by the QTL on LG 12 explaining 37%. The third QTL on LG 20 had with 8% the smallest effect. The additive effects of all three QTL had a positive sign, indicating that the presence of the 'Samourai' alleles at these QTL positions increased phytosterol content. All three QTL together explained 60% of the total genetic variance and 54% of the total phenotypic variance. For the QTL at LG 6 and LG 12, QTL for individual phytosterols were found in the same or neighbouring marker intervals (Table 3 and Figure 1). This indicates the presence of one gene at those positions simultaneously affecting individual and total phytosterol content. For the individual phytosterols between 4 and 8 QTL were detected, which together explained between 48% and 68% of the total genetic variance. The additive effects of some of the QTL had a negative sign, indicating that the presence of the 'Samourai' alleles at these QTL positions decreased phytosterol content.

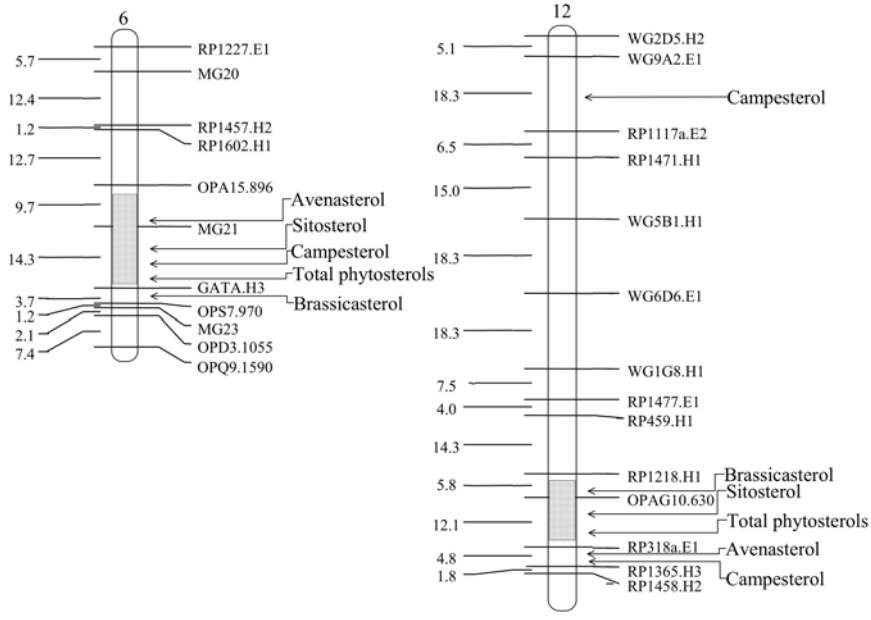

Figure 1. The framework maps of linkage groups 6 and 12 with the mapped phytosterol QTL. The distances between markers are given in cM (Kosambi 1944). The grey areas are representing the confidence intervals of 3 LOD score units around the most likely position of the erucic acid genes.

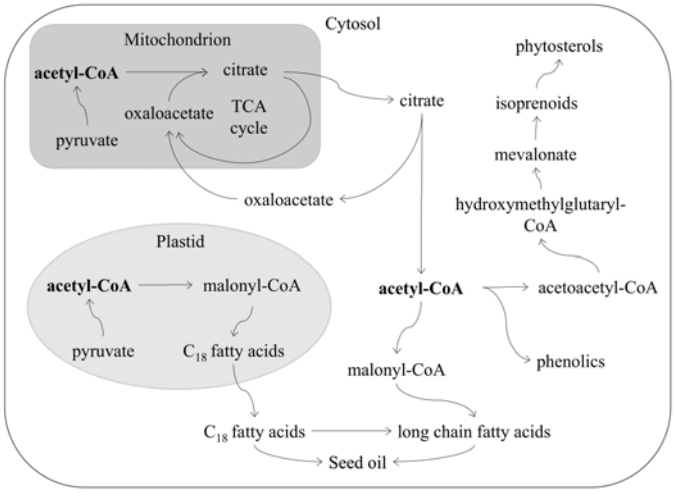

Figure 2. Biosynthetic scheme of phytosterols, fatty acids and phenolics in plants (adapted from Fatland *et al.* 2002).

The positions of the QTL for total phytosterol content and for most of the individual phytosterols on LG 6 and 12 overlapped with the positions of the two erucic acid genes (Ecke *et al*. 1995). Considering the close negative correlation between phytosterols and erucic acid (Table 2) and the fact, that cytoplasmic acetyl-CoA is a central common precursor for the

biosynthesis of phytosterols and long chain fatty acids (Figure 2), it seems likely that the two erucic acid genes exert a pleiotropic effect on phytosterol content. Cytoplasmic acetyl-CoA is also a precursor of phenolic compounds, among which sinapate esters are predominant in rapeseed. Hence, the observed negative correlation between phytosterols, erucic acid and sinapate esters (Table 2) may be caused by competition for acetyl-CoA, which obviously is available only in limited quantities.

Acknowledgments

The authors express gratitude to Uwe Ammerman for his professional technical assistance, to Mladen Radoev for his help in QTL mapping and to Dr. Kemal Gül for providing the seed samples. The research was financially supported by the Hans-Böckler-Stiftung.

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