QTL ANALYSIS OF CONDENSED TANNINS CONTENT IN *BRASSICA NAPUS* L.

ANALIZE QTL PRIVIND CONȚINUTUL ÎN TANINURI CONDENSATE DIN *BRASSICA NAPUS* L.

F. D. LIPSA[1], R. J. SNOWDON[2], W. FRIEDT[2]

[1] University of Agricultural Sciences and Veterinary Medicine Iasi
[2] Justus-Liebig University Giessen, Germany
Aleea Mihail Sadoveanu nr. 3, Iasi, 700490, Romania, E-mail: flipsa@uaiasi.ro

Abstract: *Oilseed rape/canola (Brassica napus) represents a potentially valuable source of vegetable protein due to its favourable composition of essential amino acids. However, the use of rapeseed protein for human nutrition is presently not possible due to the presence of major anti-nutritive compounds, which also reduce the value of rapeseed meal as a source of animal feed. Especially relevant in this regard are dietary fibre, dark-coloured tannins and bitter-tasting sinapate esters. Yellow coloured seeds are of particular interest for oilseed rape breeding because of their association with a thinner seed coat resulting in reduced dietary fibre and condensed tannin content. This considerably improves the feed and protein quality of rapeseed meal after oil extraction. Plant tannins make up a distinctive group of high molecular weight phenolic compounds that have the ability to complex strongly with proteins, starch, cellulose and minerals. Chemically three groups of tannins are distinguishable: phlorotannins, hydrolysable and condensed tannins (syn. proanthocyanidins, PAs). In rapeseed (Brassica napus L.) condensed tannins are largely responsible for the dark colour of the seed coat, where they accumulate predominantly in the endothelium cell layer between the outer integument and the aleuronic layer. Whereas the proportion of condensed tannins in the cotyledons of B. napus seeds is comparatively low (0.1-0.5% of dry weight), condensed tannins in dark-seeded B. napus can comprise up to 6% of the seed coat.*
The objective of this study was to identify quantitative trait loci (QTL) for seed colour, individual and total condensed tannins (syn. proanthocyanidine, PAs) content in a winter rapeseed doubled haploid (DH) population. The plant material consisted of 166 DH lines derived from a cross between an inbred line of the black-seeded German winter oilseed rape cultivar 'Express' and the true-breeding, yellow-seeded line '1012/98', both with 00-seed quality. The QTL were mapped using the software PLABQTL based on seed analyses of DH lines grown on field trials in Rauischholzhausen and Gross-Gerau (Germany). Seed colour was measured quantitatively based on digital reflectance values. Individual PAs and total flavonoid content were quantified via HPLC (High Performance Liquid Chromatography) using internal standards for quantification.

Rezumat: *Rapița (Brassica napus) reprezintă o potențială sursă de proteine vegetale, datorită compoziției sale echilibrate în aminoacizi esențiali. Culoarea galbenă a tegumentului seminal reprezintă un obiectiv important din cadrul programului de ameliorare al rapiței deoarece este un caracter asociat cu un conținut mai redus de compuşii antinutritivi (sinapină, taninuri condensate, fibră) ce reduc valoarea nutritivă a şrotului de rapiță utilizat ca sursă de hrană pentru animale. Taninurile din plante sunt compuşi fenolici care au capacitatea de a forma complexe insolubile cu proteinele, mineralele şi celuloza. Din punct de vedere chimic se disting trei tipuri: phlorotanninuri, taninuri hidrolizabile (galice) şi taninuri condensate (sin. proanthocyanidine, PAs).*
Obiectivul acestui studiu este de a realiza cartarea genetică a unei populații de rapiță pentru a identifica şi caracteriza locus-urile ce determină culoarea semințelor, conținutul total în flavonoizi, şi conținutul individual în taninuri condensate.

Key words**:** *Brassica napus, seed colour, condensed tannins, QTL mapping*
Cuvinte cheie**:** *Brassica napus, culoarea semințelor, taninuri condensate, cartare QTL*

INTRODUCTION

New varieties of oilseed rape (*Brassica napus*) that combine the yellow seed trait with low levels of anti-nutritive tannins would represent a highly significant new product that would considerably raise the value of rapeseed meal as a protein source for animal nutrition.

In rapeseed condensed tannins are largely responsible for the dark colour of the seed coat, where they accumulate predominantly in the endothelium cell layer between the outer integument and the aleuronic layer. Condensed tannins can potentially have a major impact on animal nutrition, particularly because of their ability to form indigestible, astringent or bitter-tasting complexes with proteins. One option to overcome this problem is the breeding of yellow-seeded rapeseed with reduced condensed tannins in the seed coat. This might be achievable via selection of genotypes with smaller endothelium cells and consequently a spatial reduction in condensed tannin accumulation (seed coat structural cell mutants), or alternatively by selection of genotypes with reduced biosynthesis of condensed tannins (flavonoid biosynthesis mutants).

By localising quantitative trait loci (QTL) for condensed tannin content in *B. napus* seeds and comparing these to the positions of promising candidate *tt*-genes, we hope to develop closely-linked molecular markers for selection regarding important genes involved in the accumulation of antinutritive tannins in rapeseed meal.

MATERIAL AND METHODS

Using the software JoinMap 3.0 a dense genetic map was generated from a population of 166 doubled-haploid lines derived from a cross between an inbred line of the black-seeded German winter oilseed rape cultivar 'Express' and the true-breeding, yellow-seeded line '1012/98', both with 00-seed quality. The QTL were mapped using the software PLABQTL 1.3 based on seed analyses of DH lines grown on field trials in Rauischholzhausen and Gross-Gerau (Germany). Seed colour was measured quantitatively based on digital reflectance values. Individual PAs and total flavonoid content were quantified via HPLC (High Performance Liquid Chromatography) using internal standards for quantification.

RESULTS AND DISCUSSIONS

A total of 176 polymorphic Markers (126 AFLP and 50 SSR-Marker) covering 1171 cM were localized in the genetic map for DH population (Figure 1). The linkage groups were designated based on the known marker positions using the standard N1 to N19 nomenclature for *B. napus*, with the expectation of one unidentified group that was named as KG14.

The mean size of chromosomes is 61.6 cM, and this corresponds to an average marker distance of 6.6 cM and 9 markers per chromosome. The largest linkage group (N17) with 112 cM consists of 3 AFLP plus 6 SSR markers and the smallest (N7) has 22 cM and consists of 4 AFLP plus 2 SSR markers.

Table 1 shows the chromosomal positions, flanking markers, LOD score and phenotypic effects of all putative QTL identified for seed colour and all HPLC phenolic compound peaks showing segregation within the DH mapping population.

For seed colour trait three QTL located on linkage groups N9, N11 and N15 explain 66.8% of total phenotypic variance (R^2). On linkage group N9 a major QTL for seed colour explained 40.9% of the observed partial phenotypic variance and play an important role in phenotype occurrence. At the same location on linkage group N9 were found the main QTLs for ADF (acid detergent fibber), NDF (neutral detergent fibber) and ADL content (acid detergent lignin) (B. Wittkop, IPZ Giessen, unpublished results). The second QTL (LOD = 7.6) was found on linkage group N11 and has a smaller effect (R^2 = 19.0%; part. R^2 = 19.8%) with an additive effect of 0.29%. The third QTL is characterized by the lowest LOD score for seed

color (LOD = 3.9) and is localized on linkage group N15.

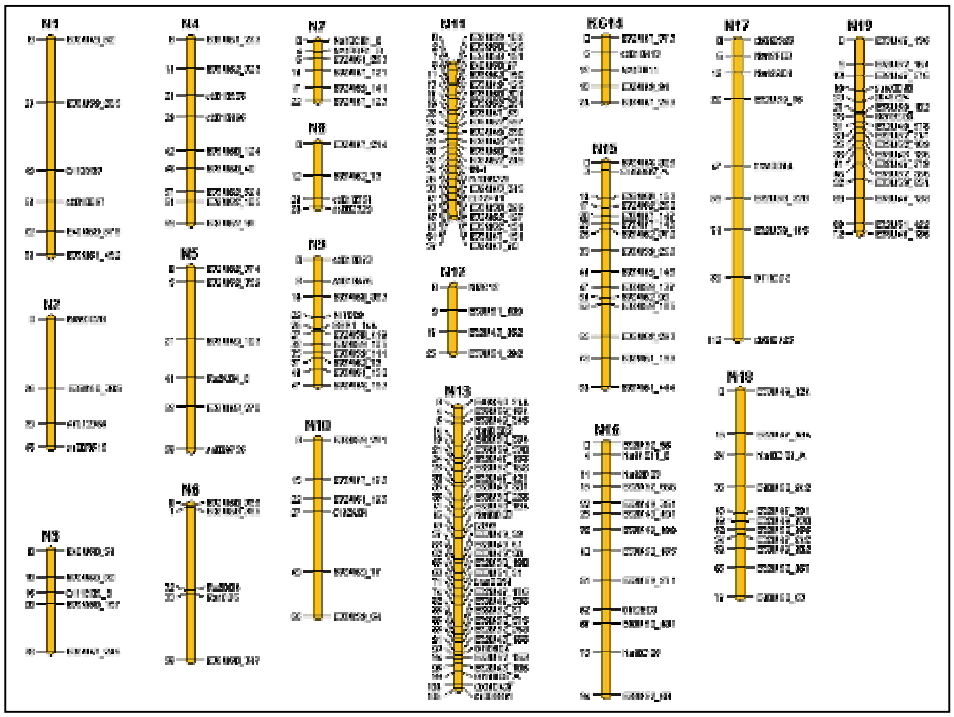

Figure 1. Genetic map of doubled haploid (DH) populations

In double haploid (DH) population for three oligomeric proanthocyanidins (F2PA2, F2PA3, F2PA6) ten QTL on five linkage groups were found. For oligomeric proanthocyanidin F2PA2 were identified five quantitative trait loci (QTL), which from 8.2 to 26.2% of phenotypic variance explained. The total phenotypic variance is therefore 38.9%. For oligomeric proanthocyanidin F2PA3, four QTLs were localized on linkage groups N11, N13, N15 and N16 with LOD values from 3.4 to 7.0 and 36.9% phenotypic variance. The most influent QTL is positioned on linkage group N11 and explain 17.7% of phenotypic variation. For oligomeric proanthocyanidin F2PA6 was a QTL on linkage group N16 near the SSR Lokus Ol12E03 detected. Two QTL for different oligomeric PAs (F2PA3 and F2PA2, respectively) were located at the same position on chromosome N13 and N15 and presumably represent gene which alters the type of oligomeric compound produced in the two parental lines. A major QTL for seed colour co-localised with a major locus for oligomeric PAs content (F2PA3) at the same position on chromosome N11. Also, two QTL for F2PA3 and F2PA6 co-localise on linkage group N16.

On DH population from five quantified polymeric proanthocyanidins we found four QTL on four different linkage groups for three of them (F3PA3, F3PA4, F3PA6). For polymeric Proanthocyanidin F3PA3 was identified a QTL on linkage group N2. This QTL explained 9.7% of the observed phenotypic variance and 6.2% of partial phenotypic variance by a LOD score of 3.6. The most influent QTL on genotypic variance for polymeric proanthocyanidin F3PA4, located near the SSR-Marker Ol12F11, explain alone 16.3% of phenotypic variance and 8.5% of partial phenotypic. This QTL was mapped in linkage group

N11. The second QTL for F3PA4 (LOD = 4.1) was placed on linkage group N19 and has a minor effect on trait variance (R^2 = 10.8%; part. R^2 = 4.1%). Both QTLs explain 12.3% from total phenotypic variance. For polymeric proanthocyanidin F3PA6 a QTL on linkage group N1 was detected. The QTL is characterized by a small LOD score (3.8) and explains 2.6% of partial phenotypic variance. The QTL for the polymeric Proanthocyanidine F3PA4 is situated on linkage group N11 in the immediate near of *tt*1 gene. Also, it is located at distance for 8 cM from a QTL for seed colour and a QTL for oligomeric PAs content (F2PA3).

Table 1

QTL for seed colour and condensed tannins in doubled haploid (DH) populations of the *B. napus*

Trait		Marker-Interval	Chr./ Position (cM)	LOD Score	R^2 (%)	Partial R^2 (%)
Seed colour		Ni4D09 / SSR1_144	N9 / 26	6,38***	16,2	40,9
		E32M62_197 / E35M62_151	N11 / 48	7,61***	19,0	19,8
		Ol10D02_A / E35M60_153	N15 / 4	3,89*	10,2	12,2
Flavonoid compounds		sn012964 / sn003616	N2 / 40	6,88***	18,2	8,4
		Ni4D09 / SSR1_144	N9 / 24	3,80*	10,0	7,1
		tt1-1 / mr000228	N11 / 36	8,44***	20,9	18,8
Oligomer PAs	F2PA2	E32M50_419 / E32M59_105	N9 / 30	10,88***	26,2	7,1
		E33M50_158 / E32M62_249	N11 / 32	3,06	8,2	10,2
		E33M49_61 / E33M49_50	N13 / 60	7,67***	19,3	18,0
		Ol10D02_A / E35M60_153	N15 / 4	6,56**	16,7	9,9
		E35M62_370 / E33M59_258	N15 / 26	8,50***	21,1	19,1
	F2PA3	E32M62_197 / E35M62_151	N11 / 48	6,98***	17,7	15,6
		E33M49_61 / E33M49_50	N13 / 62	5,21***	13,5	7,7
		E35M62_370 / E33M59_258	N15 / 26	3,36	9,0	6,4
		E33M59_271 / Ol12E03	N16 / 62	5,62***	14,5	9,9
	F2PA6	E33M59_271 / Ol12E03	N16 / 58	3,70**	9,8	7,2
Polymeric PAs	F3PA3	bras0020 / E35M60_305	N2 / 6	3,58*	9,7	6,2
	F3PA4	E32M48_245 / Ol12F11	N11 / 40	6,36***	16,3	8,5
		E32M49_578 / E32M62_247	N19 / 32	4,09*	10,8	4,1
	F3PA6	cb010097 / E40M60_578	N1 / 62	3,77*	10,0	2,6

***** significant at 0,05 level by Permutations analyse; ****** significant at 0,01 level; ******* significant at 0,001 level
R^2 (%) = phenotypic Variation, **Partial R^2 (%)** = partial phenotypic Variation, **Chr=** Chromosome

Preliminary localisation of significant QTL for total flavonoids and individual condensed tannins indicated that some loci co-localise with major QTL for seed colour that were described previously by BADANI et al. (2006). On the other hand numerous QTL were detected that do not appear to have a significant effect on seed colour but may represent

epistatic gene loci that contribute to the high environmental variation seen in yellow- and brown-seeded *B. napus* lines. By comparing the levels of flavonoid compounds with QTL it may be possible to identify genes within the flavonoid biosynthesis pathway (Routaboul et al. 2006, Lepiniec et al. 2006) that are responsible for minor differences in seed colour in *B. napus*.

The correlation of total condensed tannins content with seed colour was comparatively low (r = 0.39) and this suggests that the yellow seed colour could not be used as a selection marker for total condensed tannins content and also, that a significant proportion of the total seed flavonoids are non-coloured flavonoids (LIPSA et al. 2007). These non-coloured flavonoids do not influence the seed colour but may still have a nutritional effect by forming indigestible compounds with proteins, for example.

CONCLUSIONS

1. 176 polymorphic Markers (126 AFLP and 50 SSR-Marker) covering 1171 cM were localized in the genetic map for DH population.

2. In double haploid (DH) population we found three QTL for seed colour, three QTL for total seed flavonoids, ten QTL for oligomeric proanthocyanidins (F2PA2, F2PA3, F2PA6) and four QTL for polymeric proanthocyanidins (F3PA3, F3PA4, F3PA6).

3. Mapping of candidate genes for flavonoid biosynthesis and comparison to QTL for major flavonoid compounds will enable us to identify genes involved in the expression and control of seed colour in yellow-seeded oilseed rape.

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