

## CONDENSED TANNINS IN BRASSICA NAPUS: QTL MAPPING, CANDIDATE GENES AND ASSOCIATIONS WITH QUALITY TRAITS

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*Condensed tannins (syn. proanthocyanidins, PAs) from rapeseed meal can potentially have a negative impact on non-ruminant livestock and human 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). Both types of transparent testa (tt) mutants are well-characterised in Arabidopsis; however the genetic basis of the yellow-seed trait in the polyploid genome of rapeseed is still not completely understood.*

*As plant material 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 were used. The genetical map was constructed using AFLP and SSR markers. 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). Quality traits were measured quantitatively based on digital reflectance values. Total PAs content was via Vanillin assay quantified. Individual PAs and total flavonoid content were quantified via HPLC (High Performance Liquid Chromatography) using internal standards for quantification.*

*By localising quantitative trait loci (QTL) for condensed tannin content, seed colour and other quality traits 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 compounds in rapeseed meal.*

**Key words:** B. napus - condensed tannins - quality traits - QTL mapping

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). The phlorotannins have been isolated from species in several genera of brown algae, whereas the hydrolysable and condensed tannins are widely distributed throughout the plant kingdom. Plant tissues containing tannins include bark, wood, fruit, seeds, leaves, roots and plant galls. Different groups of tannins have been associated with the maintenance of seed dormancy, while others have allelopathic and bactericidal properties. 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 aleurone layer. Whereas the proportion of condensed tannins in the cotyledons of *B. napus* seeds is comparatively low (only 0.1-0.5% of dry weight), condensed tannins in dark-seeded *B. napus* can comprise up to 6% of the seed coat. This means that they contribute significantly to rapeseed meal, with a total content of up to 800 mg/100g after oil extraction.

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 METHOD

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, protein- and oil content were measured quantitatively based on digital reflectance values. Individual PAs and total flavonoid content were quantified via Vanillin- and HPLC- (*High Performance Liquid Chromatography*) Assays using 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. 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 (*fig.1*).



Table 1

## OTL Results for YE2-DH Population

Trait		Marker-Interval	Chr./ Position (cM)	LOD Score	R <sup>2</sup> (%)	Partial R <sup>2</sup> (%)
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
		OI10D02_A / E35M60_153	N15 / 4	3,89*	10,2	12,2
Oil content		E32M50_419 / E32M59_105	N9 / 32	4,31**	11,3	2,0
		E33M50_299 / E32M62_197	N11 / 44	3,68**	9,7	8,8
		E33M49_148 / E33M59_137	N15 / 46	4,42**	11,5	11,3
Proanthocyanidin content (Vanillin-Assay)		tt1-1 / mr000228	N11 / 36	4,51**	11,8	9,9
		E32M61_422 / E33M47_186	N19 / 72	4,32**	11,3	6,7
Flavonoid compounds (HPLC-Assay)		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
Oligomeric 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
		OI10D02_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 / OI12E03	N16 / 62	5,62***	14,5	9,9
	F2PA6	E33M59_271 / OI12E03	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 / OI12F11	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<sup>2</sup> (%) = phenotypic Variation, Partial R<sup>2</sup> (%) = partial phenotypic Variation, Chr= Chromosome

In double haploid (DH) population for three oligomeric proanthocyanidins (F2PA2, F2PA3, F2PA6) ten QTL on five linkage groups were found. For



of *Arabidopsis thaliana* represent interesting candidate genes for the analogous character in oilseed rape. The seed-specific genes *tt1* and *tt16* are involved in development of the seed endothelium, in which proanthocyanidins accumulate, and in flavonoid biosynthesis. The transcription factors *tt2* and *tt8* also regulate gene expression in the seed coat epidermis, where they are involved in the control of flavonoid biosynthetic genes such as *ban*. *tt10* determine browning of *Arabidopsis* seed coat, as result of involvement in the oxidative polymerization of flavonoids, particularly proanthocyanidins, which are polymers of flavan-3-ol subunits such as epicatechin and catechin.

New varieties of oilseed rape that combine the yellow seed trait with low levels of antinutritive compounds would represent a highly significant new product that would considerably raise the value of rapeseed meal as a source of valuable vegetable protein for food and feed purposes.

## 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 oil content, two QTL for total Pas content, three QTL for total seed flavonoids, ten QTL for oligomeric proanthocyanidins (F2PA2, F2PA3, F2PA6) and four QTL for polymeric proanthocyanidins (F3PA3, F3PA4, F3PA6).

3. 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.

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## IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES AND STEMS FROM EUROPEAN MISTLETOE (*VISCUM ALBUM*)

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*The aim of this study was to determine in vitro, the antioxidant activity of different aqueous extracts of V. album, depending on the host trees using ferric reducing power (FRAP) assay and Folin Ciocalteu assay. When we compared the antioxidant activity of leaves and stems, it can be observed that the highest antioxidant activity was recorded in the case of stems. The values obtained by FRAP assay, varied from  $0.23 \pm 0.03$  mg/l vitamin C equivalent / g of fresh leaves for the VAJ extract to  $0.52 \pm 0.05$  mg/l vitamin C equivalent / g of fresh leaves for the VAM extracts. Similarly results were obtained in the case of stems extracts ( $0.54 \pm 0.20$  mg/l vitamin C equivalent / g of stem for the VAJ extract to  $0.90 \pm 0.40$  mg/l vitamin C equivalent / g of stem for the VAS extracts). The influence of the host tree may play a very significant part in the assessment of the mistletoe as a plant raw material for phytopharmaceutical formulas. We also reported for the first time the significant antioxidant potential of different plant parts of mistletoe (stems versus leaves), stems being more concentrated in antioxidants, being more protected from sun irradiation than leaves.*

**Key words:** *Viscum album*, FRAP assay, antioxidant, phenolic

Aqueous extracts of the European mistletoe have been widely used for decades as alternative treatment and adjuvant cancer therapy, particularly in Germany, Austria and Switzerland.

European mistletoe (*Viscum album* L., family Santalaceae) is an evergreen, semiparasitic plant, normally found growing on a variety of trees, especially pine, poplar, apple trees, locus trees etc. Although there are many varieties of mistletoe, including the American (*Phorandendron serotinum* or *Phorandendron flavescens*), the European (*Viscum album* L.), and the Korean (*Viscum album* L. *coloratum*), most investigative work has been done on European mistletoe. A number of