Metabolic engineering of the sinapic acid ester content in oilseed rape (*Brassica napus* L.)

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ABSTRACT

In the *Brassicaceae* family, sinapic acid esters (SAE) are important anti-nutritional compounds. They contribute to the bitter taste and astringency of rapeseed products. In addition, SAE may form complexes with proteins, thus lowering the nutritional value of the protein products. A substantial reduction of SAE in oilseed rape would improve its use as a protein crop. *Brassica napus* accumulates two major sinapic acid esters in seeds: sinapoylglucose and sinapine. Sinapic acid is converted to sinapoylglucose by UDP-glucose:sinapic acid glucosyltransferase (SGT) and then to sinapine by sinapoylglucose:choline sinapoyltransferase (SCT). This work focuses on the *Agrobacterium tumefaciens* mediated transformation with a ds-RNA-interference construct deduced from sequence of the key enzyme SGT. This should result in a substantial decrease of sinapoylglucose and sinapine.

KEYWORDS: *Brassica napus,* SGT, sinapoylglucose, sinapine, ds-RNA-interference construct

INTRODUCTION

In the *Brassicaceae* family, sinapic acid esters (SAE) are present in the seeds and constitute in the case of *Brassica napus*, 1-2% of the seed dry matter. Sinapic acid esters are associated with the bitter taste, odor and taste deterioration, and toxic effects as it damages the thyroid, liver and kidneys of the cattle. A substantial reduction of SAE in oilseed rape would improve its use as a protein crop. *Brassica napus* and other members of the *Brassicaceae* accumulate three major sinapic acid esters, sinapoylglucose, sinapine and sinapoylmalate. Leaves contain only sinapoylmalate, whereas seeds accumulate sinapine and smaller amounts of sinapoylglucose as well as of some other not yet fully characterized sinapic acid containing compounds. Sinapic acid (Fig.1) is converted to sinapoylglucose by UDP-glucose:sinapic acid glucosyltransferase (SGT) and then to sinapine by sinapoylglucose:choline sinapoyltransferase (SCT). The gene encoding SGT is the first obvious target for suppressing sinapic acid ester synthesis; the full-length cDNA of the SGT gene has been isolated (Milkowski et al., 2000) and a 200 bp-sequence (Fig. 2) was inserted as a inverted repeat in a ds-RNA-interference construct for *Agrobacterium* mediated transformation.

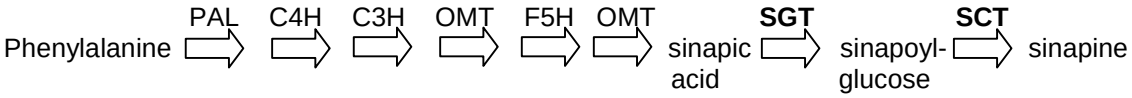

Fig.1. The Pathway of Sinapic acid Ester Biosynthesis

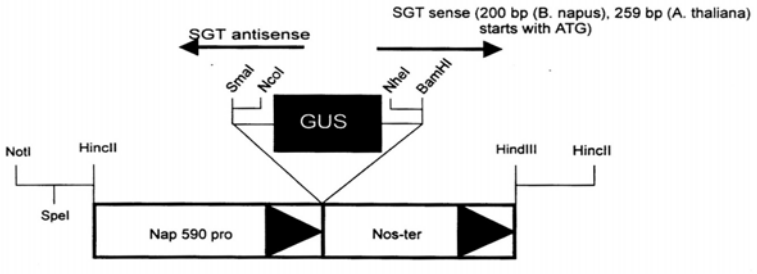

Fig. 2. SGT-ds-RNA-interference construct

MATERIAL AND METHODS

Standard techniques were used for the *Agrobacterium tumefaciens* mediated transformation (Moloney et al., 1989). Hypocotyls (*Brassica napus* cv. Drakkar) were infected with the *Agrobacterium* strain AGL0, then transformants were selected on a solidified medium in two steps containing PPT (5 mg/l; 10 mg/l). Regenerated plants were tested by a PPT-ELISA-Test (Steffens, Ebringen) and screened by PCR, using specific primers for the SGT construct. Primary transgenic plants (T_1) were selfed in the greenhouse. 23 different seeds of cv. Drakkar were germinated in vitro and were used as controls and regenerated in the same way like transgenic plants. T_2 seeds (4-10 g) harvested from regenerated T_1 plants were scanned on a monochromator NIR Systems model 6500 (NIR Systems, Inc., Silver Springs, MD, USA) equipped with sample autochanger. The sample size scanned was about 3 g intact seeds. For each sample the sinapic acid ester content was calcutated by NIRS calibrations for sinapoylglucose, sinapine, sum of remaining not fully characterized SA containing compounds and total SAE content calculated as sinapic acid in mg/g seeds (zum Felde et al., 2003). For the estimation of oil, protein and glucosinolates (GSL) the NIRS calibration raps 2001.eqa was used.

RESULTS AND DISCUSSION

The 23 regenerated transgenic T1-plants were normal in growth and morphology in comparison with the controls and there was no difference in other important agronomic seed traits, like oil, protein and glucosinolate (GSL) content (Tab.1). The transgenic plants had varying levels of all 3 SAE components. The variation for the sum of all sinapic acids esters is shown in Fig. 3.

Tab.1. Comparison (mean ± SD) of agronomic traits between controls and transgenic lines

	n	Oil (%)	Protein (%)	GSL (µmol/g seeds)
Control cv. Drakkar	23	44.89 ± 2.37	24.71 ± 1.97	22.35 ± 2.13
Transgenic lines	23	45.20 ± 2.64	24.30 ± 2.42	20.80 ± 3.77

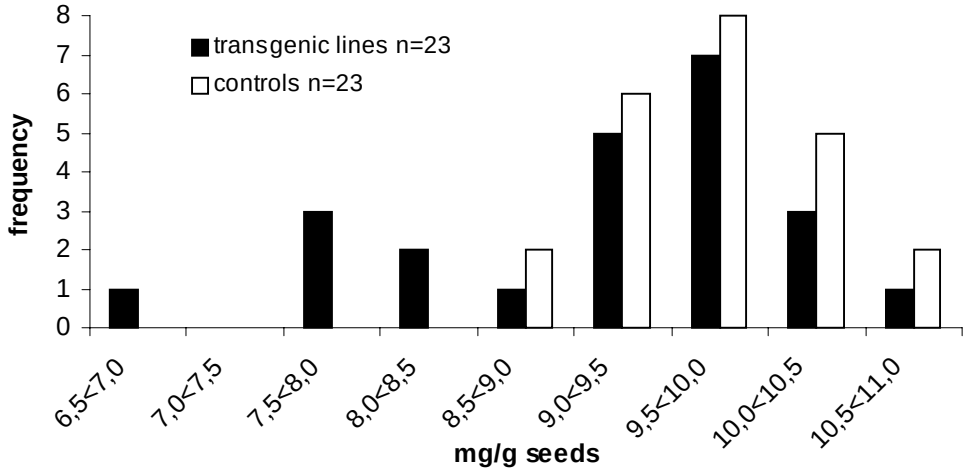

Fig.3. Distribution of frequency for the total SAE content (mg/g) in T_2 seeds of lines transformed with the SGT-ds-RNA-interference construct and controls

Some of the transgenic plants (T_1) showed in the seeds a 39% reduction in the sinapoylglucose content in comparison with the control plant having the least sinapoylglucose content (Tab. 2). The sinapine content in the fully mature seeds showed a reduction by only 13%.

Table 2. Total amount (mg/g) and relative reduction (%) of SAE compounds in the T_2 seeds of *B. napus* lines transformed with SGT-ds-RNA-interference construct and controls

	Controls (min)	Transgenic lines (min)	
	mg/g	mg/g	%[2]
Sinapoylglucose	1.68	1.03	39
Sinapine	6.65	5.73	13
Sum of not fully characterized SAE[1]	1.34	0.82	39
Total SAE content[1]	8.80	6.74	24

[1] calculated as sinapic acid min = minimum

[2] reduction in % of control (=100%)

Among the transgenic lines a high and significant correlation was found between sinapoylglucose and the sum of not fully characterized SAE, but there was no correlation between sinapoylglucose and sinapine (Tab. 3). Similar results were found by zum Felde (pers. communication) in a DH-Population segregating for SAE content.

Table 3. Correlation between sinapoyl compounds in the T_2 seeds of primary transgenic *B. napus* lines with SGT-ds-RNA-interference construct

	Sinapoylglucose	Sinapine	Sum of not fully characterized SAE
Sinapine	-0.08		
Sum of not fully characterized SAE[1]	0.77**	0.17	
Total SAE content	0.67**	0.52**	0.86**

The results obtained by using NIRS are so far preliminary. HPLC analysis of the T2 seeds are in progress to obtain more accurate results. Nevertheless, the results indicate a significant reduction in sinapoylglucose and the sum of not fully characterized SAE. Further reduction can be expected by selection of homozygous T2 plants in the next generation.

Since no significant reduction of sinapine was found in the T2-seeds, this leaves open some questions on how sinapic acid ester synthesis is regulated in *Brassica napus*. One speculation is that the SCT is a rate limiting enzyme in the sinapine synthesis. Then, a reduction in sinapine can only be expected if a more drastical suppression of the sinapoylglucose synthesis is achieved. Furthermore, the SGT could be a regulatory point for the sum of not fully characterized SAE, because this compound is highly correlated with sinapoylglucose, i.e. reduction in sinapoylglucose results in reduced not fully charaterized SAE contents.

ACKNOWLEDGEMENTS

We thank Rosi Clemens and Nicole Ritgen for excellent technical support. This project is a part of the BMBF project `NAPUS 2000-Healthy Food from Transgenic Rape Seeds`. The financial support of the UFOP (Union for the Promotion of Oil and Protein Plants e.V., Bonn, Germany) for attending this conference is appreciated.

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