Inheritance of phytosterol content and oil content in a doubled haploid population derived from the winter oilseed rape cross Sansibar x Oase

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ABSTRACT
Improving the seed quality of oilseed rape is an important issue in breeding programmes. In a previous screening, the two winter oilseed rape cultivars Sansibar and Oase were shown to be quite different with respect to their oil and phytosterol content. Sansibar has high phytosterol content and a low oil content, and Oase vice versa. With the aim to study the inheritance of these two traits, a doubled haploid population with n=253 lines were generated from F_1 plants Sansibar x Oase. The doubled haploid population was cultivated in 2009/10 in a field experiment at the experimental station Göttingen-Reinshof. At maturity the main raceme of ten open pollinated plants per DH line were harvested and bulked for analysis. Preliminary results from the first year field experiment and from 140 lines with respect to the inheritance of seed oil and phytosterol content are reported.

INTRODUCTION
Phytosterols are one of the minor seed constituents that could increase the value of the oilseed rape crop. They are well known for their low-density-lipoprotein (LDL) cholesterol lowering effects (Best et al. 1954) and have been incorporated by the food industry as bioactive components to develop functional food products, particularly in margarine and dairy products. Total phytosterol content in crude rapeseed oil ranges from 0.5 to 1.0%, making it one of the richest natural sources of phytosterol (Piironen et al. 2000). Recent study by Amar et al. (2008a) detected three QTL for phytosterol content in 148 doubled haploid (DH) lines of winter rapeseed. Two large QTL were mapped within the confidence intervals of the two erucic acid genes while the third QTL was only a minor one. The result suggested a pleiotropic effect of the two erucic acid genes on phytosterol content and that the population used, segregating for erucic acid content, was deemed to be not suitable to identify QTL for individual and total phytosterol content and to analyze interaction with other seed quality traits apart from erucic acid. On the other hand, the relationship between phytosterol content and oil content remains inconclusive as contradictory results were observed in three DH populations analyzed by Amar et al. (2008b). In a collection of 27 modern winter oilseed rape cultivars, however, highly contrasting phytosterol and oil contents were found. One of the cultivars with the lowest phytosterol content (3580 mg/kg), Oase, had the highest oil content while the cultivar with the highest total phytosterol content (4800 mg/kg), Sansibar, had the lowest oil content (Amar et al. 2009). These two cultivars were selected as parents to develop DH lines segregating for phytosterol and oil content with the objective to investigate the inheritance of phytosterol and oil content.

MATERIALS AND METHODS
Plant material and field experiment: The plant material consisted of 253 DH lines derived from F_1 plants of the cross between 00-quality winter oilseed rape cultivars Sansibar and Oase (Amar et al. 2009). The DH population along with their two parental lines was grown in a field experiment without replicate at Göttingen-Reinshof, in the growing season 2009/2010. Within the population the two parental lines were replicated 11 times at equal distances. Bulked seeds from the main raceme of ten open pollinated plants were used for trait analyses.
Analysis of phytosterols content and other quality traits: The total phytosterol content and its composition were analyzed with gas-liquid chromatography (GLC) using a simplified sample preparation and derivatization method developed by Amar et al. (2008b). The individual phytosterols (mg $100g^{-1}$ seed) determined were brassicasterol, campesterol, stigmasterol, sitosterol and avenasterol. Seed oil content (%), protein content (%), and glucosinolate content (µmol g^{-1}) were determined with near-infrared reflectance spectroscopy (NIRS) using the equation raps2010.eqa developed by Tillmann (2010), adjusted to 9% moisture. Fatty acid composition of the seed oil was analyzed with gas-liquid chromatography (GLC). This include oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), expressed as % of the sum of all fatty acids.

Data analysis: Results presented here are from a subsample of 140 DH lines and 5 replicates of the two parental lines that were analyzed up to date for phytosterol content and other quality traits. Statistical analyses were performed using STATISTICA software version 9.1 (StatSoft, 2010).

RESULTS AND DISCUSSIONS

The individual phytosterols determined by GLC were brassicasterol, campesterol, stigmasterol, sitosterol and avenasterol (Fig. 1). These five individual phytosterols are the terminal product sterols classified as 4-desmethyl sterols, the predominant sterol class in vegetable oil. The total phytosterol content determined in the subsample of 140 DH lines ranged from 293 – 492 mg $100g^{-1}$ seed, with a mean concentration of 403 mg $100g^{-1}$ seed. Sitosterol is the predominant sterol in oilseed rape, with a mean concentration of 193 mg $100g^{-1}$ seed, followed by campesterol (139 mg $100g^{-1}$ seed), and brassicasterol, with a mean of 51 mg $100g^{-1}$ seed. Avenasterol and stigmasterol were present in lower concentration, 13.6 mg $100g^{-1}$ seed and 7.8 mg $100g^{-1}$ seed, respectively but showed the largest coefficient of variance as compared to the three major sterols. The relative percentage composition of the individual sterols is similar to studies reported from Warner and Mounts (1990), Hamama et al. (2003), and Amar et al. (2008b), even though the total amount of phytosterol varied.

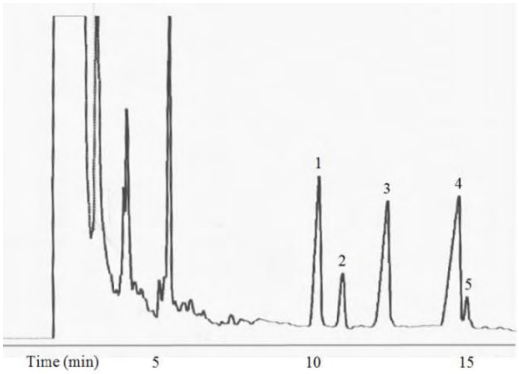

Fig. 1: Representative gas chromato-gram of the individual phytosterol determined in seed sample. Peak numbers indicate 1 = cholesterol (internal standard), 2 = brassicasterol, 3 = campesterol, 4 = stigmasterol, 5 = sitosterol, and 6 = avenasterol.

The mean of the total phytosterol concentration for the two parental lines were 423 mg $100g^{-1}$ seed for Sansibar and 368 mg $100g^{-1}$ seed for Oase, which fall within the range of the DH population (Fig. 2). As reported by Amar et al. (2009), Sansibar which has a higher concentration of total phytosterols showed a lower oil content than Oase. On the contrary, positive correlation between total phytosterol content and oil content was highly significant in the DH population while negative correlation was observed between total phytosterol content and protein content.

Positive correlation was highly significant between total phytosterol content and all of the individual phytosterols except that of brassicasterol which was significant at P = 0.05. Campesterol and sitosterol showed the strongest correlation with total phytosterol content which could be explained by their major contributing factor to the total phytosterol content in the seed. Brassicasterol, the common sterol for rapeseed and other *Cruciferaceae*, showed a higher significant correlation with protein content and glucosinolate content than with total phytosterol content. This could mean that canola quality rapeseed has the added advantage of being high in phytosterol content. In addition to that, highly significant positive correlation was observed between sitosterol and oleic acid while negative correlations were observed between sitosterol and linoleic acid, and between sitosterol and linolenic acid. The results is in contrast with what Abidi et al. (1999) had reported that there was no significant correlation between fatty acid composition and the distribution of phytosterols although the phytosterol composition was found markedly affected by genetic modification on canola oils.

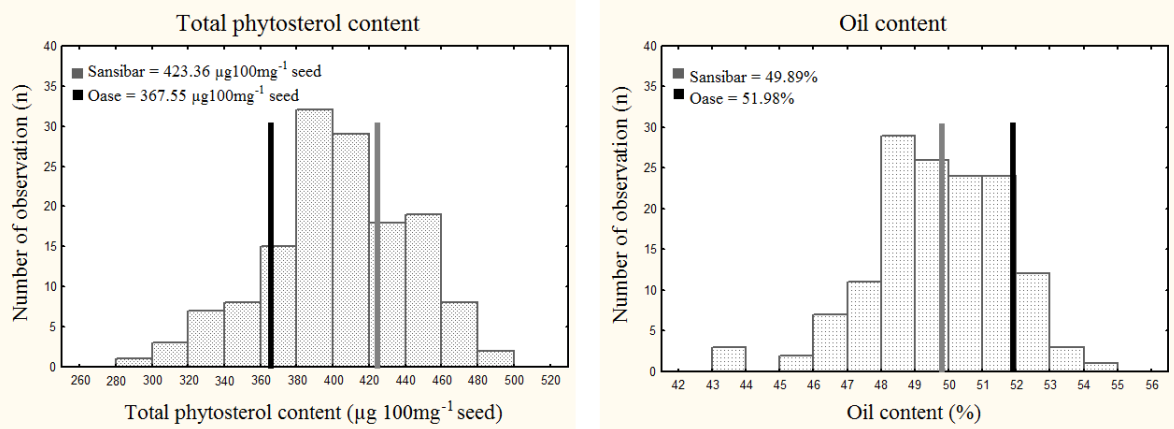

Fig. 2: Frequency distribution of the total phytosterol content (left) and oil content (right) in a subsample of 140 DH lines derived from a cross between the cultivar Sansibar and Oase

Tab. 1: Descriptive statistics of phytosterol content and other quality traits in the two parental lines and DH population.

Traits	Unit	Parents		DH population (n=140)				
		Sansibar (n=5)	Oase (n=5)					
		Mean		Mean	Range (min – max)		Std dev.	CV[a]
Brassicasterol	mg 100g^{-1} seed	51.4	50.6	50.5	31.7	- 61.3	4.4	8.8
Campesterol		144.6	125.2	138.6	84.8	- 191	22.0	16.0
Stigmasterol		8.0	6.8	7.8	0	- 15.4	2.8	36.4
Sitosterol		205.1	172.8	192.9	130	- 237	19.0	9.8
Avenasterol		14.3	12.1	13.6	4.8	- 38.5	4.9	36.7
Total phytosterol		423.4	367.6	403.3	293	- 492	39.8	9.9
Oil	%	49.9	52.0	49.8	43.4	- 54.8	1.9	3.9
Protein	%	18.3	19.4	19.9	16.5	- 26.5	1.6	8.2
Glucosinolate	µmol g^{-1}	22.6	24.3	25.9	14.8	- 41.8	4.7	18.2
C18:1	%	62.8	63.2	62.9	57.6	- 68.4	2.1	3.3
C18:2	%	20.5	19.9	20.4	16.0	- 24.2	1.5	7.1
C18:3	%	7.9	7.7	7.7	5.3	- 10.3	0.9	12.2

[a]CV = Coefficient of variation

Tab. 2: Spearman's rank correlation coefficient (r_s) between different seed quality traits in the DH population (n=140)

	Brassicasterol	Campesterol	Stigmasterol	Sitosterol	Avenasterol	Total phytosterol	Oil	Protein	Glucosinolate	C81:1	C18:2
Brassicasterol	1										
Campesterol	0.07	1									
Stigmasterol	-0.01	0.07	1								
Sitosterol	0.08	0.49 **	0.30 **	1							
Avenasterol	0.04	0.67 **	-0.01	0.31 **	1						
Total phytosterol	0.18 *	0.89 **	0.25 **	0.79 **	0.65 **	1					
Oil	0.01	0.35 **	-0.18 *	0.30 **	0.32 **	0.35 **	1				
Protein	-0.24 **	-0.24 **	0.18 *	-0.14	-0.19 *	-0.22 **	-0.79 **	1			
Glucosinolate	-0.23 **	-0.03	0.17 *	0.01	-0.19 *	-0.03	-0.34 **	0.51 **	1		
C18:1	-0.21 *	0.09	0.13	0.29 **	0.01	0.17 *	0.30 **	-0.14	0.14	1	
C18:2	0.09	-0.06	-0.07	-0.25 **	-0.02	-0.14	-0.27 **	0.11	-0.11	-0.86 **	1
C18:3	0.23 **	-0.07	-0.09	-0.22 **	0.06	-0.11	-0.15	0.12	-0.08	-0.64 **	0.37 **

*significant at $P = 0.05$

**significant at $P = 0.01$

CONCLUSIONS

The preliminary results from a subsample of 140 DH lines developed from the winter oilseed rape cross Sansibar × Oase showed a substantial variation for mapping of quantitative trait loci (QTL) for individual and total phytosterol content as well as to investigate the correlation with other important quality traits.

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