Genetic variation of bioactive compounds in doubled haploid populations of winter oilseed rape (Brassica napus L.)

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Introduction

Double low oilseed rape (Brassica napus L.) and other Brassica crops are well suited for agricultural production of high quality edible oil and proteins. In addition, Brassica napus oil and seeds are a very valuable source of natural bioactive components which take part in quenching of free radicals during metabolic processes also affect the stability of oil to prevent the rapid oxidation of fatty acids. They are the main factor of cell membranes maintenance in their proper structure and permeability. The representatives of such components are native antioxidants – tocochromanols: tocopherols, tocotrienols which are vitamin E-active compounds, plastochromanol-8 (PC-8) and phytosterols, β-carotene as well as phenolic compounds. One of the directions of oilseed rape breeding is the increase of the amount of these antioxidant components in seeds (Bartkowiak-Broda et al.2008; Amar et al., 2008; Marwede et al., 2004).

The preliminary study on the content of compounds possessing antioxidative character has been carried out on two populations of doubled haploid lines (DH) derived from F_1 hybrids resulting from reciprocal crosses of yellow and black seeded winter oilseed rape (Brassica napus L.).

Materials and Methods

Two populations o DH lines were developed from two F_1 hybrids from the reciprocal crosses of black-seed (DH $H_2$26) and yellow- seed (DH Z-114) winter oilseed rape. The biological materials consist of 63 different DH lines and two parental lines. The seeds for analysis were harvested from plants which grew on the experimental fields as described (Szala et al. 2011).
Tocopherols (α, β, γ and δ), PC-8 and β-carotene were qualitatively and quantitatively identified using HPLC. The fluorometric detector worked at excitation □=290 nm and emision □=330 nm for tocochromanols. β-carotene content was analyzed by UV-Vis spectrophotometry detector (450 nm). Phytosterols: brassicasterol, campasterol, stigmasterol β-sitosterol and avenasterol were isolated by GC. The content of total phenolic compounds (TPC) in methanolic extracts was determined by the Folin-Ciocalteu method. Separation and identification of free phenolic acids in extracts of oilseed rape carried out by HPLC after pre-cleaning techniques trial SPE.
Results are presented as means ± standard deviation from three replicates of each experiment.
P-value < 0.05 was used to denote significant differences among mean values determined by the analysis of variance (ANOVA) with the assistance of statistical package Statistica 7.1 (StatSoft, Inc., Tulsa, OK) software.

Results and Discussion

The aim of the study was qualitative and quantitative analysis of native tocochromanols phytosterols, total phenolic compounds, β-carotene in two population of DH lines obtained from reciprocal crosses yellow- and black-seeded winter oilseed rape. In the conditions of the experiments it was shown that the range of variability of tocopherols and plastochromanol-8 in tested DH lines of two populations in excess of the value of parental lines. There were no direct crosses influenced on the content of tocopherols and the PC-8 in the seeds of DH lines (table 1). The content of α-T β-T, γ-T, δ-T and PC-8 differ statistically significant in these populations. Designated factor α-T / γ-T for the two populations of DH lines ranged from 0,66 to 1,09. It was observed no significant differences between the coefficients in the two study populations, HZ and ZH. In the study observed that the ratio of α-T / γ-T in the studied DH lines surpassed the value of doubled haploid lines indicated by Marverde, 2004, (Marverde et al. 2004).

TABLE 1

Tocopherols content in seeds of two populations of doubled haploid lines and parental lines: DH H_2 26 and DH Z-114 of winter oilseed rape (Brassica napus) (mg/100 g of seeds)

	α-T	β-T	γ-T	δ-T	Total tocopherols	α-T / γ-T	PC-8
HZ (black × yellow) population — 27 DHs							
Minimum	20,2	0,05	23,8	0,32	46,2	0,66	5,0
Maximum	26,7	0,24	35,4	0,77	59,5	1,09	8,8
Coefficient of variability	8,15	34,19	8,37	17,9	5,76	11,9	14,1
ZH (yellow × black) population — 36 DHs							
Minimum	19,5	0,05	24,9	0,34	46,0	0,66	4,8
Maximum	29,6	0,41	33,8	0,63	60,2	1,08	9,8
Coefficient of variability	9,56	52,27	7,68	17,32	6,08	12,30	15,72
Parental lines							
DH $H_2$26 (black seed)	23,6	0,19	29,2	0,45	53,4	0,81	5,8
DH Z114 (yellow seed)	28,3	0,18	23,5	0,56	52,5	1,21	8,1

On the basis of data presented in the table 2, it was found that the amount of sterols in all seeds tested DH lines ranged from 210.7 to 432.7 mg/100 g of seeds. The content of total sterols in the parental lines of DH $H_2$26 (367.5 mg/100g) and DH Z114 (341.7 mg/100g) were similar (table 2). The average content of sterols in the population of HZ was higher than the doubled haploid population ZH. The dominant sterol in the rape seeds was β-sitosterol, the percentage is from 46.3% to 56.7%. Further sterols are campesterol from 25.1% to 34.2%, more than 11.8% brassicasterol to 17.1% and avenasterol from 0,8 to 4.8%. Smallest share attributable to stigmasterol between 0.3% and 3.8% (table 2). Examined the contents of sterols in winter oilseed rape DH lines were little higher than with those showed Amar et al 2008. The large genotypic differences for the total and individual phytosterol content indicate that effective selection for high phytosterol genotype in a cultivar development program would be possible.

Analysing the composition of the seed of parental lines of two populations of winter oilseed rape in terms of β-carotene, found that the line DH Z114 is the richest source of this compound (an average of 0.32 mg/100g) and DH line $H_2$26 was characterized by a lower content (mean value 0,22 mg/100g). For both study populations no DH line which showed a higher content of β-carotene than the parental yellow-seeded line (table 3). Comparing β-carotene content in both populations showed that the average content of this compound was higher in DH lines derived from F_1 hybrids derived from crosses between lines DH Z114 x DH $H_2$26 than DH $H_2$26 x DH Z114. The direction of the crossing had a importance on the content of β-carotene content in DH lines.

TABLE 2

Phytosterols composition and total phytosterols in seeds of two populations of doubled haploid lines and parental lines: DH H_2 26 and DH Z-114 of winter oilseed rape (Brassica napus)

	Phytosterols (% in total)					Total phyto-sterols mg/100 g
	Brassicasterol	Campasterol	Stigmasterol	β-Sitosterol	Avenasterol	
HZ (black x yellow) population – 27 DHs						
Min	11,8	25,8	0,3	48,8	0,8	291,5
Max	16,5	34,2	3,8	54,7	4,0	432,7
ZH (yellow x black) population – 36 DHs						
Min	12,6	25,1	0,0	46,3	1,1	210,7
Max	17,1	33,7	3,2	56,7	4,8	418,2
Parental lines						
DH $H_2$26 (black seed)	13,27	33,00	1,4	50,6	1,7	367,5
DH Z114 (yellow seed)	15,48	25,8	0,3	56,5	2,00	341,7

The extract from seed samples of genetically different doubled haploid lines of winter oilseed rape demonstrated large variation in total phenolic compounds (table 4). Doubled haploids lines obtained from reciprocal crosses of black seed - DH $H_2$26 and yellow seed – DH Z114 Brassica napus exceeded the value of two parents in total phenolic compounds and total phenolic acids content. The content of phenolic compounds in the DH lines from both populations exceeded the value early specified for var. Kronos 2965.7 mg/100 g which, as demonstrated Siger, 2004, which was characterized by a high content of these compounds (Siger et al 2004).

TABLE 3

β-caroten content in seeds of two populations of DH lines and parental lines: DH H_2 26 and DH Z-114 of winter oilseed rape (Brassica napus)

	β-caroten content in mg/100g			Coefficient of variation
	Mean	Min	Max	
HZ (black x yellow) population - 27 DHs	0,21	0,15	0,29	18,00
ZH (yellow x black) population - 36 DHs	0,21	0,13	0,32	20,43
DH $H_2$26 (black seed)	0,22	0,18	0,25	11,46
DH Z114 (yellow seed)	0,32	0,26	0,38	13,78

The dominant phenolic acid in rapeseed is sinapic acid nearly (20%). Other protocatechic acid, p-hydroxybenzoic, vanillic, caffeic, p-coumaric and ferulic occurred in small amounts, usually their contents were not higher than 2 mg/100g. While the participation of sinapic acid derivative was 70%.

TABLE 4

Total phenolic compounds and total phenolic acids content in seed extracts of two populations of DH lines and parental lines: DH H_2 26 and DH Z-114 of winter oilseed rape (Brassica napus)

	Total phenolic compounds (mg/100g seeds)			Total phenolic acids (mg/100g seeds)		
	Mean	Min	Max	Mean	Min	Max
HZ (black x yellow) population - 27 DHs	2422,8	1914,1 ±10,1	2856,0 ±30,3	49,73	35,82 ±0,20	65,18 ±0,58
ZH (yellow x black) population - 36 DHs	2391,9	1886 ±96,0	2794 ±50,5	49,79	35,82 ±1,05	64,73 ±1,31
DH $H_2$26 (black seeded)	2022,6			44,99		
DH Z114 (yellow seeded)	2234,1			40,86		

Considering the range of genetic variation of bioactive compounds among doubled haploids of two populations, selected DH lines may be good parents for further breeding programs focused on the improving the quality of oilseed rape oil.

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