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Changes in Phytosterols in Rapeseed (*Brassica napus* L.) and Their Interaction with Nitrogen Fertilization

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ABSTRACT

Phytosterols occur in relatively high concentration in the seeds of rapeseed (*Brassica napus* L.) and are affected by environmental and cultural factors. Nitrogen is one of the most important factors in rapeseed production. The aims of this reserach were to determine phytosterol levels in some new rapeseed varieties and to investigate interactions between phytosterol levels and nitrogen fertilization, in the growing season of 2004 - 05 on 19 newrapeseed varieties. The identification of phytosterols were achieved using capillary column gas-chromatography instrument. Data revealed that sitosterol (1078.8 - 1972.5 mg kg^{-1}) was the major component, followed by campesterol (1004.7 - 1598.1 mg kg^{-1}). Under N-fertilizer application, sitosterol content changed between 727.9 and 2104.7 mg kg^{-1}, followed by campasterol ranged from 642.2 to 1539.0 mg kg^{-1}. Total phytosterols content ranged between 3220 - 4147.3 mg kg^{-1} for N_0 level; 2423.0 and 4198.4 mg kg^{-1} for N_1. Significant interactions between genotype and nitrogen fertilization were found for the second major component campasterol and total phytosterol content. Rapeseed is rich on phytosterols. But according to high nitrogen fertilization, phytosterol content can be decreased significantly.

Key Words: Quality; Phytosterol; Rapeseed; Interaction; Nitrogen

INTRODUCTION

Oilseed rape is one of the important oil-producing crop worldwide. Its oil is rich in non-saturated fatty acids, tocopherols and phytosteroles. Phytosterols are composed of 17 carbon atoms with a characteristic three-dimensional arrangement of four rings. They play a great role in human nutrition and lower LDL cholesterol (Gylling *et al*., 1997; Nissinen *et al*., 2002; Trautwein *et al*., 2003). Phytosterols are well-studied components and 40 different phytosterols have been found in higher plants, but its content is affected by environmental conditions. Brassicasterol is a typical phytosterol for *Brassicaceae* family including rapeseed (Benveniste, 2002). Amar (2004) reported genotype x environment interactions for phytosterols in rapeseed. Nitrogen (N) fertilization may increase rapeseed yield significantly, while causing a decrease in seed-oil content (Gül, 2002). Phytosterols are predominantly found in the oil; therefore, greater oil content is important to get high level of phytosterols (Becker *et al*., 1999).

Gordon and Miller (1996) investigated 10 different oil types from corn, rapeseed, groundnut, olive, soybean, safflower, oleic sunflower, linoleic sunflower, cottonseed and palm oil. They have found that rapeseed is one of the highest phytosterol suppliers (6900 mg kg^{-1}). Also, Vlahakis and Hazebroek (2000) compared rapeseed, sunflower and soybean for phytosterols. In their study, rapeseed contained approximately twice the amount of total phytosterols (4590 - 8070 mg kg^{-1}) compared to sunflower (2100 - 4540 mg kg^{-1}) or soybean (2340 - 4660 mg kg^{-1}) oils. In addition to genotypic variation and environmental effect, the changed genetic architecture can play a great role for phytosterol variation. Abidi *et al*. (1999) reported differences in total phytosterol content, caused by genetic modifications. In their study, comparing transgenic and non-transgenic rapeseed genotypes differing in fatty acid composition, these authors concluded that the phytosterol content was influenced by the genetic modification in fatty acid composition. A significant decrease in the amount of three major phytosterols (sitosterol, campesterol & brassicasterol) was observed in non-transgenic rapeseed varieties grown for low-linolenic and high-oleic acid. In addition, the amount of brassicasterol varied widely based on genotype and growing conditions.

The above information suggests that there is no specific study on interaction between rapeseed varieties and nitrogen fertilization. Nitrogen is one of the major inputs in rapeseed production and contributes to changes in seed oil and quality. The aims of this reserach were: (1) to determine phytosterol levels in some new rapeseed varieties and (2) to investigate interactions between phytosterol levels and nitrogen fertilization.

MATERIALS AND METHODS

Plant materials and field experiments. Eighteen new varieties along with a standard genotype (Licrown) of rapeseed kindly provided by KWS Saat AG and NPZ Saatzucht, Germany were sown in the field during 2004 - 05 growing season at the Experimental Station of Onsekiz Mart

University, Çanakkale. The experiment was laid out in completely randomized block design with 3 replications. Plots consisted of four rows were 1.20 m x 5 m in size. The N_0 plots were not fertilized and the N_1 plots were fertilized with 130 kg ha^{-1} nitrogen (N); 1/3 of N was applied with planting and the remaining was in March, between the rows. Plants were harvested manually and all plants from a plot were threshed together. Samples were taken randomly from each plot for phytosterol analyses.

Sample preparation for capillary gas liquid chromatography. The rapeseed samples were prepared and analysed as described by Aitzetmüller *et al.* (1998) and Amar (2004). All samples were analyzed at the Institute for Plant Breeding and Plant Production of the University Göttingen, Germany using a gas chromatography capillary gas-liquid chromatographer (Perkin-Elmer 8420, San Jose, California, USA) with a flame ionization detector and split/split less injector. Medium polarity capillary column was DB-5 15 m long, with 0.25 mm outer diameter and 0.1 mm inner diameter, coated with 5%-phenyl 95%-methylpolysiloxane of 0.5 µm stationary phase thickness (J & W Scientific, USA). Hydrogen was used as a carrier gas as described by Dutta and Normen (1998). Identification of phytosterols in the sample material was based on their retention time. The phytosterol standards were commercially available and identified on GC under the defined conditions (Fiebig *et al*., 1998).

Phytosterols, brassicasterol (BRS), campesterol (CAS), stigmasterol (STIS), sitosterol (SITS), avenasterol (AVS) and total phytosterol in seed (TPC) for rapeseed were investigated. Also the steryl esters were included in the total phytosterols content. The variance analysis was done using general linear model of SAS (SAS Institute Inc., 1999) statistical software and means were compared.

RESULTS

The mean values of nitrogen levels showed significant differences for all traits (Table I). The values for BRS were significantly different. The BRS content of the investigated rapeseed genotypes was the highest in H602014 and H602016, while the lowest in Adder and Aragon. Among the varieties, Adder had the lowest values for CAS, SITS and TPC. The CAS content in Express was significantly higher than the other varieties. Baros had the highest value for SITS and TPC (Table I).

Nitrogen fertilization produced significant differences in the investigated traits (Table II). H602014, H602016 and Viking had the highest values for BRS content, which was the lowest in Express. However, Express had the highest value of CAS and STIS content. For all varieties STS content ranged between 1078.8 and 2117.9 mg kg^{-1}, whilst this range was very high for AVS. TPC content for Baros was significantly higher than the other varieties. The lowest value for TPC was observed for Artus (Table II). On the contrary, at N_1 level, no significant differences were discernible for BRS (Table III). The values for CAS ranged between 642.2 (Adder) and 1539.0 (Artus) mg kg^{-1}, showing great differences among the varieties. Except of STS, Adder and Artus had the lowest and the highest values, respectively for all traits (Table III).

The variance analyses were done to find the interaction between genotype and nitrogen fertilization (Table IV). Except for CAS and TPC, genotypes x nitrogen interactions for other traits were not evident. Variance analysis revealed a significant genotype x nitrogen interaction on CAS content ($p = 0.000$). Similar results were obtained for TPC ($p = 0.008$).

Table I. Comparison of 19 rapeseed genotypes for phytosterol contents in two N levels (N_0 = 0 kg ha^{-1}, N_1 = 130 kg ha^{-1})

	Phytosterols (mg kg^{-1})					
Genotyp	**BRS**	**CAS**	**STS**	**SITS**	**AVS**	**TPC**
Talent	415.63 abcd	1267.3 bcdef	4.867 bc	1562.1 bcde	323.7 abcd	3573.5 bcde
Aragon	350.75 d	1410.2 abcd	3.920 bc	1700.8 bcd	320.9 abcd	3786.6 abcd
Elan	400.04 abcd	1239.5 cdef	4.527 bc	1724.6 abcd	275.9 bcd	3644.5 bcde
Rasmus	460.58 abc	1213.0 defg	5.862 ab	1197.7 e	579.0 a	3456.1 cdef
Viking	470.03 ab	1472.1 ab	5.257 abc	1680.1 bcd	191.9 cd	3986.1 ab
Express	382.26 abcd	1554.1 a	7.208 a	1703.0 abcd	228.2 bcd	3874.7 abc
Alesi	373.83 bcd	1236.9 cdef	4.788 bc	1838.2 abc	167.5 cd	3621.3 bcde
Triangle	403.92 abcd	1083.7 fg	3.940 bc	1692.0 bcd	290.5 abcd	3474.0 cdef
Adder	345.57 d	1016.2 g	4.880 abc	1210.2 e	522.5 ab	3099.4 f
Licrown	398.85 abcd	1220.5 defg	4.688 bc	1403.2 de	424.3 abc	3451.5 cdef
H602016	485.91 a	1153.8 efg	4.672 cb	1648.4 bcd	380.8 abcd	3673.6 bcde
H602014	486.69 a	1156.2 efg	5.437 abc	1639.7 bcd	194.3 cd	3482.4 cdef
Prince	405.54 abcd	1404.3 abcd	4.350 bc	1812.3 abcd	161.4 cd	3787.9 abcd
Action	420.88 abcd	1157.9 efg	4.007 bc	1436.9 cde	265.0 bcd	3284.7 ef
Artus	391.81 abcd	1271.9 bcdef	3.492 c	1735.3 abcd	307.0 abcd	3709.4 bcde
Titan	358.23 cd	1203.1 defg	4.477 bc	1632.7 bcd	170.0 cd	3368.5 def
Mendel	439.57 abcd	1317.4 bcde	4.457 bc	1577.1 bcde	236.2 bcd	3574.7 bcde
Baros	417.63 abcd	1441.6 abc	5.387 abc	2111.3 a	173.1 bcd	4149.1 a
Tenno	411.63 abcd	1304.6 bcde	4.885 abc	1704.9 abcd	114.2 d	3540.2 cde
LSD 5%	109.08	220.45	2.33	410.49	295.35	432.75

Means within a column followed by the same letter are not significantly different at the $p = 0.05$ level

Table II. Mean phytosterol values of each N level

	Phytosterols (mg kg^{-1})					
N Level	**Bras**	**Cam**	**Stig**	**Sito**	**Ave**	**Total**
N_0	411.55 ns	1292.98 ns	4.62 ns	1769. 15 a	322.06 b	3700.35 a
N_1	419.26 ns	1246.43 ns	4.97 ns	1495.14 b	356.12 a	3521.92 b
LSD 5%	33.12	71.86	0.74	136.74	105.11	142.67

Means within a column followed by the same letter are not significantly different at the p = 0.05 level

DISCUSSION

In this study, the total phytosterol content, on seed basis, for rapeseed genotypes varied between 3220 and 4147.3 and 4198 mg kg^{-1} at N_0 and N_1, respectively (Table I - III). Considering there is about 45% oil in rapeseed, total phytosterols on oil basis would be almost double. Then, our values would be comparable with Gordon and Miller's (1997), who reported a total phytosterol content of 6900 mg kg^{-1} from 5 rapeseed varieties. Some values of total phytosterol contents from varieties are clearly higher compared to previous studies. Such differences could be explained by genetic and environmental factors or analysing conditions.

Baros contained higher phytosterol levels than most of the varieties at both nitrogen levels. Artus contained the lowest phytosterol level at N_0, but a 30% increase was observed when fertilized with nitrogen, which was an

Table III. Comparison of 19 rapeseed genotypes for phytosterol contents in N0 level (0 kg ha^{-1})

	Phytosterols (mg kg^{-1})					
Genotyp	**Bras**	**Cam**	**Stig**	**Sit**	**Ave**	**Total**
Talent	422.67 abc	1368.8 abcdef	4.447 b	1775.0 abcd	97.1 d	3668.0 bcde
Aragon	353.66 bc	1543.6 ab	3.300 b	1940.9 abc	103.8 cd	3945.3 abc
Elan	393.40 abc	1366.1 abcdef	4.127 b	1972.5 a	98.4 d	3834.6 abcde
Rasmus	462.07 abc	1093.1 gf	3.790 b	1078.8 e	748.6 a	3386.4 ef
Viking	473.95 a	1413.9 abcde	4.437 b	1676.5 bcd	266.0 bcd	3834.8 abcd
Express	335.44 c	1598.1 a	8.447 a	2005.1 ab	119.8 cd	4066.9 ab
Alesi	380.67 abc	1259.1 bcdef	4.527 b	1944.2 abc	139.0 cd	3727.4 abcde
Triangle	405.45 abc	1014.5 g	3.920 b	1781.7 abcd	204.2 cd	3409.8 def
Adder	346.77 abc	1390.3 abcdef	5.010 b	1692.6 bcd	341.1 bcd	3775.7 abcde
Licrown	403.75 abc	1221.9 cdefg	4.720 b	1597.6 bcd	424.2 bc	3652.1 bcde
H602016	481.75 a	1104.6 efg	4.120 b	1473.5 de	547.2 ab	3611.2 cdef
H602014	488.23 a	1199.1 cdefg	4.973 b	1965.7 ab	95.2 d	3753.3 abcde
Prince	390.26 abc	1394.0 abcdef	3.977 b	1811.8 abcd	118.8 cd	3718.8 bcde
Action	451.07 abc	1171.4 dfg	4.063 b	1684.5 bcd	199.5 cd	3510.5 def
Artus	355.55 bc	1004.7 g	3.673 b	1528.8 cd	327.8 bcd	3220.5 f
Titan	438.09 abc	1315.7 abcdefg	4.960 b	1839.4 abcd	99.8 d	3698.0 bcde
Mendel	454.31 ab	1227.9 bcdefg	4.397 b	1910.0 abc	69.9 d	3666.5 bcde
Baros	394.08 abc	1503.3 abc	5.880 ab	2117.9 a	126.1 cd	4147.3 a
Tenno	388.21 abc	1376.6 abcdef	4.930 b	1817.3 abcd	92.5 d	3679.5 bcde
LSD 5%	117.07	316.6	3.28	421.08	320.89	426.41

Means within a column followed by the same letter are not significantly different at the p = 0.05 level

Table IV. Comparison of 19 rapeseed genotypes for phytosterol contents in N_1 level (130 kg ha^{-1})

	Phytosterols (mg kg^{-1})					
Genotyp	**Bras**	**Cam**	**Stig**	**Sit**	**Ave**	**Total**
Talent	408.59	1165.8 def	5.287 ab	1349.2 bcd	550.3 ab	3479.1 abcd
Aragon	347.84	1276.9 bcdef	4.540 b	1460.6 abc	538.0 ab	3627.9 abcd
Elan	406.68	1112.8 def	4.927 ab	1476.7 abc	453.3 ab	3454.3 bcd
Rasmus	459.08	1332.9 bcdef	7.933 a	1316.6 bcd	409.4 ab	3525.8 abcd
Viking	466.11	1530.3 ab	6.077 ab	1683.8 abc	451.1 ab	4137.4 ab
Express	429.08	1510.0 abc	5.970 ab	1401.0 bc	336.5 ab	3682.5 abcd
Alesi	366.98	1214.8 bcdef	5.050 ab	1732.3 abc	196.0 ab	3515.1 abcd
Triangle	402.39	1152.8 def	3.960 b	1602.2 abc	376.8 ab	3538.2 abcd
Adder	344.38	642.2 g	4.750 ab	727.9 d	703.9 a	2423.0 e
Licrown	393.95	1219.1 bcdef	4.657 ab	1208.9 cd	424.3 ab	3250.9 cd
H602016	490.07	1203.1 cdef	5.223 ab	1823.4 abc	214.3 ab	3736.1 abcd
H602014	485.15	1113.3 def	5.900 ab	1313.8 bcd	293.4 ab	3211.5 cd
Prince	420.82	1414.7 abcd	4.723 ab	1812.7 abc	204.1 ab	3857.0 abc
Action	390.68	1144.4 def	3.950 b	1189.4 cd	330.5 ab	3059.0 de
Artus	428.07	1539.0 a	3.310 b	1941.8 ab	286.2 ab	4198.4 a
Titan	425.03	1090.5 ef	3.993 b	1426.1 bc	240.1 ab	3185.7 cd
Mendel	424.84	1407.0 bcde	4.517 b	1244.2 cd	402.4 ab	3483.0 abcd
Baros	441.17	1380.0 bcde	4.893 ab	2104.7 a	220.2 ab	4150.9 ab
Tenno	435.05	1232.6 bcdef	4.840 ab	1592.4 abc	135.8 b	3400.8 cd
LSD 5%	164.9	322.5	3.28	647.02	537.1	734.89

Means within a column followed by the same letter are not significantly different at the p = 0.05 level

exception for Artus (Table I). The mean values of total phytosterol contents were significantly higher for N_0 (3700.35) than for N_1 (3521.92), thereby forming the basis of significant differences under both N conditions. Sitosterol was the major component with high contribution to total phytosterol content followed by campesterol in rapeseed (Table IV). Thus, the variation in phytosterol content was mainly due to the variations in sitosterol and campesterol contents. The contribution of single components to total phytosterol and their values were consistent with the previous studies (Appelqvist *et al*., 1981; Gordon *et al*., 1997; Piironen *et al*., 2000; Amar, 2004). The contribution of avenasterol to total phytosterol content is not important but the variation was great at N_0 level (Table III).

Nitrogen fertilization decreased total phytosterol content significantly. Gül (2002) reported that nitrogen fetilization increased the seed yield and total oil yield from a certain field although decreased the oil concentration in the seed. Our study indicated nitrogen fertilization had a negative effect on total phytosterol content and single components (Table II). Other environmental effects have been reported in different species. Yang *et al*. (2003) compared phytosterol levels in seeds of two *Vaccinium* (berries) species, grown at two locations in northern and southern Finland. They found differences within genotypes and explained it by geographic and climatic conditions. In contrast, Määttä *et al*. (1999) investigated seven oat cultivars grown at three different locations in Sweden and reported significant differences in total phytosterol content among cultivars, but no effect was found for the growing location. The effect of nitrogen on oil yield and fatty acids are known. However, there is no information about the N effect on the phytosterol content. Because of the negative effects of nitrogen on oil and fatty acids, an adverse effect on phytosterol can be expected. In view of this prediction, interaction between genotype and nitrogen fertilization were significant for CAS and TPC. The individual contribution of the major component CAS to TPC is important. Thus the interaction between genotype and nitrogen fertilization for TPC can be explained by high contribution of CAS. However, abandoning nitrogen fertilization is not a reasonable way. To obtain the best yields, it is important to know the individual chracteristics of the genotypes under the applied fertilizers effects.

Our results indicated that genotypes differed in their reaction to nitrogen fertilization. Some varieties e.g., Baros, were rather stable and not affected by the fertilization. Stability over different environmental conditions is a desired character for plant production. The highly significant genotypic differences for phytosterol content and composition may be used for breeding of new varieties with improved phytosterol content. However, genetic variation found in the present study is relatively limited. Analysing a larger number of genotypes in different locations and conditions should allow the identification of a much higher variability, which could then be used for breeding of improved cultivars with high and stable phytosterol content. Nevertheless, growing high oil-producing varieties would increase the phytosterol yield in a field.

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