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Full Length Research Paper

Fatty acid composition of *Camelina sativa* as affected by combined nitrogen and sulphur fertilisation

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Camelina (false flax) oil is an important source of linolenic acid (C18:3). As agronomic treatments such as fertilization may affect seed quality parameters in oilseeds, fatty acid composition and oil content of *Camelina sativa* were investigated as affected by the combined application of nitrogen and sulphur in pot experiments. Nitrogen was applied as NH_4NO_3 at rates of 0.6 (N_1), 0.9 (N_2) or 1.2 (N_3) g per pot. To increase the natural soil S-SO_4^{2-} level of 25 mg/kg (S_0) to 35 mg/kg (S_1), 45 mg/kg (S_2) and 55 mg/kg (S_3) were supplied as $(NH_4)_2SO_4$. Results of this study show that seed oil content ranged from 37.01 to 41.23% of seed dry matter, and oil content was significantly reduced by N fertilizer application. The contents of palmitic acid (range 6.9 to 11.0%), oleic acid (range 12.8 to 16.3%) as well as arachidic and 11, 13–icosadienoic acids were also affected by fertilisation, whereas variations in linolenic acid content were not significantly influenced by the fertilizer treatments applied.

Key words: Nitrogen, sulphur, fertilizer, *Camelina sativa*, seed, fatty acid, GC-FID.

INTRODUCTION

Camelina (false flax, gold-of-pleasure, *Camelina sativa* [L.] Crtz.) belongs to the Brassicaceae family and is a flexible oilseed crop that can be grown under different climatic and soil conditions (Zubr, 2003). Agronomic interest in camelina as an oilseed crop is due to its low-input characteristics (Putnam et al., 1993). While its agronomic features have been considered to be quite acceptable, the oil content has been described as being in the low range of 320 to 440 g/kg seed dry matter (Vollmann et al., 1996; Zubr, 2003). Therefore, improvement of the seed oil content would undoubtedly enhance the competitiveness of camelina in comparison with other oilseed crops (Vollmann et al., 2005). In many oilseed crops such as soybean, oilseed rape or linseed, selection for low linolenic acid content has been practised (Burton et al., 2004) in order to improve shelf life of the oil and to reduce the rate of trans fatty acid formation during oil processing, which has a negative health effect (Stender and Dyerberg, 2004).

However, as a polyunsaturated fatty acid (PUFA) α-linolenic acid is a nutritionally valuable omega-3 fatty acid, reducing the incidence of cardiovascular disease as well as various other health risks in humans (Ruxton et al., 2007). Thus, vegetable oil from camelina appears as an important source of omega-3-fatty acids due to its high concentrations of linolenic acid (Hrastar et al., 2009) and good oxidative stability (Abramovic and Abram, 2005). The consumption of camelina oil can contribute to the desirable improvement of general health of the population (Zubr, 1997; Zubr and Matthäus, 2002; Rokka et al., 2002; Lu and Kang, 2008), and significantly reduces

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Table 1. Experimental layout of the nitrogen/sulphur fertilization experiments and oil content (%) as influenced by N-S-treatments (Lošák et al., 2010, 2011).

Treatment no.	Description	N – dose (g/pot)	S-SO_4^{2-} content in soil (mg/kg)	Oil content mean ± S.D.
1	N_1S_0	0.6	25	40.02 ± 3.569^{ad}
2	N_1S_1	0.6	35	40.49 ± 4.554^{ac}
3	N_1S_2	0.6	45	39.64 ± 6.382^{ae}
4	N_1S_3	0.6	55	41.23 ± 3.108^{a}
5	N_2S_0	0.9	25	39.05 ± 2.607^{ab}
6	N_2S_1	0.9	35	38.19 ± 2.876^{bcde}
7	N_2S_2	0.9	45	37.86 ± 2.054^{bde}
8	N_2S_3	0.9	55	38.84 ± 2.638^{ab}
9	N_3S_0	1.2	25	37.26 ± 4.638^{be}
10	N_3S_1	1.2	35	37.70 ± 4.726^{bde}
11	N_3S_2	1.2	45	37.01 ± 4.912^{b}
12	N_3S_3	1.2	55	37.72 ± 0.983^{bde}

$^{a-e}$ Means followed by the same letter in the column are not significantly different (P ≤ 0.05). S.D. – standard deviation.

serum cholesterol (Karvonen et al., 2002). Of all agricultural methods, balanced nutrition and fertilisation of camelina in particular predetermines the seed quality, oil yields and composition of fatty acids (Honermeier and Agegnehu, 1996; Zadernowski et al., 1999). Nitrogen is one of the most important nutrients involved in the production of oilseed crops (Urbaniak et al., 2008; Nuttall and Malhi, 1991). A number of research projects have evaluated N requirements in camelina with variable results.

According to studies conducted in Europe (Zubr, 1997), camelina can be successfully grown with N levels of 100 kg N/ha. Applications of nitrogen affect yield components (Agegnehu and Honermeier, 1997), seed yield, oil and protein content and fatty acid composition (Szczebiot, 2002; Urbaniak et al., 2008; Zheljazkov et al., 2008). Sulphur deficiency inhibits the plant use efficiency of N from fertilisers (Schnug et al., 1993) and may therefore increase N losses. If the sulphur supply is insufficient, increasing rates of nitrogen will intensify this shortage (Janzen and Bettany, 1984) and further reduce yields. The aim of the present study was to explore the effect of combined N and S fertilisation on fatty acid composition and oil content of camelina.

MATERIALS AND METHODS

A pot trial with 4 replications of every treatment was set up outdoors on 20 March 2005, at the experimental site of Mendel University. A total of 6-L Mitscherlich pots were filled with 6 kg of fresh medium-heavy soil characterised as a fluvial. The pots were watered with demineralised water to a level of 60% of the maximum water-holding capacity and were kept free of weeds. The layout of the fertilizer treatments is shown in Table 1. On 18 July 2005, four plants from each pot were harvested at the stage of full maturity and the oil content and fatty acids composition of camelina seeds were determined. Soil analyses and plant analyses were published in our previous studies (Lošák et al., 2010, 2011).

Oil content

Sub-samples of about 15 g of dry seeds were analysed for oil content by near-infrared reflectance spectroscopy (NIRS) using an InfraAlyzer model 450 spectrometer and IDAS calibration software (Bran & Luebbe, Norderstedt, Germany). NIRS calibrations with validation r^2 values of 0.90 for oil content had previously been developed with reference samples from eight different environments. All seed oil content data were expressed as % of oven-dried seed dry matter.

Chemicals

Used chemicals as Dimethylformamide (DMF) puriss, disodium hydrogen citrate purum, sodium methoxide, methanol p.a., 1,4-dioxane p.a., n-hexane p.a. were purchased from Sigma-Aldrich. Food Industry FAME Mix standard was acquired from Restek.

Determination of fatty acids (FA)

Determination of fatty acids was performed according to Suter et al. (1997). Briefly, 1 g of homogenous sample of camelina was accurately weighed. Then, the sample was heated in DMF: 2.5 ml of DMF were added and the slurry refluxed whilst it was stirring, for 15 min, as pretreatment before transesterification. Samples were cooled to ambient temperature and after that transesterification followed. Firstly, 5 ml of 1,4-dioxane was added and mixed to dissolve any solid fat. Then, 5 ml of methanol containing 5% methoxide were added (vortexed for 3 s) and allowed to stand for 90 s. Consequently, 25 ml of hexane were admixed. Immediately afterwards, the reaction (saponification) was stopped by addition of 10 ml of 15% disodium hydrogen citrate in water, reducing the pH to 7-8. The hexane phase was transfered to the 50 ml volumetric flask and 10 µl of cyclohexanone as an internal standard were added, and the volumetric flask was filled to the mark.

The hexane solution was used for GC-FID analysis, which was

Table 2. Fatty acid concentrations (%) of camelina oil depending on the fertilizer treatment.

Treatment	C16:0 mean ± S.D.	C18:0 mean ± S.D.	C18:1 mean ± S.D.	C18:2 mean ± S.D.	C18:3 mean ± S.D.	C20:0 mean ± S.D.	C20:1 mean ± S.D.	C20:2 mean ± S.D.
N_1S_0	7.78^{bc}± 0.417	2.99^{a}± 0.133	14.73^{ab}± 1.356	19.49^{a}± 1.341	34.69^{a}± 3.130	1.94^{bc}± 0.084	16.34^{a}± 0.617	2.03^{ab}± 0.139
N_1S_1	7.08^{cd}± 0.081	2.80^{a}± 0.021	14.86^{ab}± 0.257	19.79^{a}± 0.256	35.32^{a}± 1.090	1.96^{ab}± 0.024	16.14^{a}± 0.313	2.04^{ab}± 0.048
N_1S_2	7.53^{bc}± 0.237	2.92^{a}± 0.166	14.96^{ab}± 0.245	19.69^{a}± 0.265	34.52^{a}± 1.862	2.07^{ab}± 0.069	16.27^{a}± 0.137	2.04^{ab}± 0.049
N_1S_3	6.98^{c}± 0.305	2.85^{a}± 0.057	15.50^{ac}± 0.337	18.70^{a}± 0.218	35.67^{a}± 0.620	1.98^{ab}± 0.057	16.41^{a}± 0.148	1.91^{ab}± 0.079
N_2S_0	8.13^{b}± 0.145	2.98^{a}± 0.174	14.32^{ab}± 0.098	18.22^{a}± 2.298	36.45^{a}± 0.237	1.91^{b}± 0.219	15.78^{a}± 0.349	2.21^{a}± 0.182
N_2S_1	6.91^{c}± 0.426	2.83^{a}± 0.212	14.37^{ab}± 0.383	19.90^{a}± 0.197	35.26^{a}± 0.732	2.12^{ab}± 0.152	16.50^{a}± 0.052	2.11^{ab}± 0.101
N_2S_2	11.02^{a}± 0.310	3.22^{a}± 0.210	16.26^{a}± 0.239	18.42^{a}± 0.842	32.02^{a}± 0.502	2.08^{ab}± 0.070	15.15^{a}± 0.284	1.83^{ab}± 0.015
N_2S_3	7.67^{bc}± 0.336	3.30^{a}± 0.328	16.16^{a}± 0.629	19.54^{a}± 1.968	32.88^{a}± 4.050	2.02^{ab}± 0.152	16.42^{a}± 0.792	2.01^{ab}± 0.122
N_3S_0	7.92^{bd}± 0.081	3.15^{a}± 0.075	15.17^{ac}± 0.359	19.24^{a}± 1.263	34.25^{a}± 3.088	2.32^{ac}± 0.076	15.94^{a}± 1.202	2.01^{ab}± 0.222
N_3S_1	7.70^{bc}± 0.216	3.17^{a}± 0.116	12.82^{b}± 1.201	19.71^{a}± 0.723	37.49^{a}± 0.290	2.15^{ab}± 0.120	15.26^{a}± 1.163	1.70^{b}± 0.135
N_3S_2	7.33^{bc}± 0.133	3.02^{a}± 0.087	13.54^{bc}± 0.069	19.45^{a}± 1.344	36.62^{a}± 1.141	2.34^{a}± 0.086	15.94^{a}± 0.943	1.75^{ab}± 0.042
N_3S_3	7.98^{bd}± 0.343	3.30^{a}± 0.236	13.86^{bc}± 0.776	20.26^{a}± 1.008	34.80^{a}± 0.422	2.26^{ab}± 0.132	15.46^{a}± 1.123	2.10^{ab}± 0.263

$^{a-d}$ Means followed by the same letter in each column are not significantly different (P ≤ 0.05). S.D. – standard deviation.

performed with Shimadzu GC 2010 using L × I.D. 30 m × 0.25 mm, d_f 0.20 µm capillary column SPB-PUFA. The injection volume was 1.0 µl with split ratio 1:100. During injection, the oven temperature was 50°C (1 min), then programmed at 20°C/min to 220°C; the total GC programme took about 30 min. Nitrogen 5.0 served as the mobile phase. Software GC solution was used for data analysis. Quantitative analysis of FA in camelina samples was accomplished by comparison with FAME Mix standard. The conversion from FAMEs to fatty acids was performed using coefficient, which was calculated as the ratio of the molecular weight of fatty acid to the molecular weight of fatty acid methyl ester according to Pimentel et al. (2010).

Statistical analyses

The data were statistically analysed according to Zapletal et al. (2009) using the statistical programme Statistica CZ 9.0. (StatSoft, Inc., 2011), using analysis of variance (ANOVA) with N rate and S rate as fixed effects on fatty acid composition. The parametric Tukey's HSD test as a post hoc test was used for the determination of significant differences. Statistically significant differences were tested at P ≤ 0.05.

RESULTS AND DISCUSSION

Oil content

In the present study, the seed oil content ranged from 37.01 to 41.23% of seed dry matter (Table 1). This range corresponded to that reports in a number of previous studies (Vollmann et al., 1996, 2005; Zubr, 1997, 2003; Agegnehu and Honermeier, 1997). In our experiment (Table 1), the oil content significantly decreased with increasing rate of N fertilisation (N_1, N_2 and N_3). The highest N dose significantly reduced the oil content of seeds in comparison to the lowest one applied with the same S rate (N_3S_0 compared with N_1S_0) or (N_3S_2 compared with N_1S_2). This finding confirmed the conclusions of Agegnehu and Honermeier (1997), who discovered that increasing the N doses from 0 to 120 kg N ha^{-1} at a seed rate of 400 seeds m^{-2} decreased the oil content of camelina from 41 to 39%. Urbaniak et al. (2008) arrived at the same conclusion. Rathke et al. (2005) suggested that this may be a conesquence of reduced availability of carbohydrates for oil synthesis with increasing N availability. In our experiment, S fertilisation did not affect the oil content of camelina.

Fatty acid (FA) composition

The results of the FA analysis of camelina seed oil are presented in Table 2. Generally, the analysis confirmed the known specific content of FA in camelina oil which was similar, but not the same as report for oils from camelina cultivated in the USA, Central and North Europe (Bonjean and

Le Goffic, 1999; Zubr and Matthäus, 2002; Abramovic and Abram, 2005; Peiretti and Meineri, 2007). The results (Table 2) showed that nitrogen/sulphur fertilization significantly influenced the concentration of palmitic (C16:0), oleic (C18:1), arachidic (C20:0) and 11, 13 – icosadienoic acid (C20:2). The content of palmitic acid ranged from 6.9±0.43% by N_2S_1 application to 11.0±0.31% in the case of N_2S_2 application. The fertilizer combinations N_2S_2 and N_2S_3 caused the highest contents of oleic acid (16.3±0.24%, 16.2±0.63%, respectively) while the lowest amount was determined in case of N_3S_1 application (12.8±1.20%). The content of arachidic acid was highest after N_3S_0 or N_3S_2 treatments, rising to 2.3±0.08 and 2.3±0.09%, respectively, in contrast to the combinations N_1S_0 and N_2S_0 (1.9±0.08% and 1.9±0.22%). Significant differences were also found between N_2S_0 and N_3S_1 for 11, 13 – icosadienoic acid (2.2±0.18% and 1.7±0.14%, respectively). The present findings are confirmed by Abramovic and Abram (2005) who find that the differences in the composition of the FA in seed oil can be caused not only by the different cultivars, but also by environmental conditions. Zubr (2003) similarly concluded that variations are due to the combined effects of climatic and soil conditions on the crop.

Conclusion

Camelina is described as a crop requiring fewer inputs than other oilseed species and producing higher amounts of α-linolenic acid. This study revealed an effect of nitrogen/sulphur fertilizer application on oil content and FA composition. The highest N dose significantly reduced the oil content of seeds in comparison to the lowest one applied with the same S rate. The N_2S_2 fertilization caused the highest contents of both palmitic and oleic acids. The concentration of linolenic acid, which is the important fatty acid with respect to human nutrition, remained unaffected by the fertilizer treatments applied. With the view of seed yield, oil yield (Lošák et al., 2010, 2011) and fatty acids composition we could recommend for *C. sativa* growing, the N_2S_2 fertilization, which means combination of 0.9 g/pot (approximately equal to 95 $kg \cdot ha^{-1}$) of nitrogen and 45 mg/kg of $S\text{-}SO_4^{2-}$.

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