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Characterization of Oil and Fatty Acid Composition in Seed Produced by Canola Regrowth

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Abstract: Canola (*Brassica napus* L.) and its relatives are known to regrow after harvesting of the foliage. However, yield and oil characteristics of seed harvested from regrowth are unknown. An experiment was conducted to obtain such information. Pre-flowering foliage was harvested from 48 plots (cut plots) grown in virginia during 2001-02 season. A group of 48 companion plots (uncut plots) was left unharvested. Upon harvesting of foliage, these plots received a factorial combination of four rates each of P and K fertilizers (0, 50, 100 and 200 kg ha^{-1}). Both sets of plots had previously received 100 kg ha^{-1} each of N, P and K. All 96 plots were harvested at maturity and data on seed yield and oil characteristics were recorded. The results indicated that seed yield from regrowth was 67% of uncut plots (1349 vs. 2020 kg ha^{-1}). The oil content in regrowth plots was significantly lower than that in uncut plots (34.7 vs. 37.1%). However, the oil from regrowth plots was considered healthier since, it contained less saturated and more unsaturated fatty acids. Effects of P and K fertilizers on canola regrowth were variable. An additional experiment with a factorial combination of three rates each of N, P and K (0, 50, 100 kg ha^{-1}) applied to the cut plots indicated that N rate effects were non-significant whereas increasing rates of P and K increased C18:0 and C18:3 fatty acids. Earlier research in virginia has established that pre-flowering canola foliage can be harvested to yield 11 Mg ha^{-1} of fresh greens and 1 Mg ha^{-1} of dry matter. Present results demonstrate the potential of canola as a dual purpose crop to enhance the income of small farmers. It could be harvested before flowering as a value-added feed/food product and the regrowth could be harvested for seeds to provide edible oil.

Key words: *Brassica napus*, nutrition, oil content, MUFA, PUFA

INTRODUCTION

Rapeseed (*Brassica napus* L. and *B. rapa* L.) crops are among the oldest cultivated plants known to man (Prakash, 1980) . In India, *B. rapa* is mentioned in ancient sanskrit literature from ca. 1500 BC and seed of *B. juncea* have been found in archeological sites dating back to 2300 BC (Prakash, 1980). Rapeseed production has a long history in China, the oldest archaeological discoveries date back to ca. 5000 BC (Raymer, 2002). Historically, *B. rapa* seems to have the widest distribution of *Brassica* oilseeds. At least 2000 years ago, it was distributed from Northern Europe and Korea, with primary center of diversity in the Himalayan Region. *Brassica napus* has probably developed in the Mediterranean area. Its production probably started in Europe during the middle ages. *Brassica napus* was introduced to Asia during the 19th century (Hegde, 1976). Canola (*Brassica napus* L.), a member of the mustard family, is receiving increased worldwide attention as a source of healthy edible oil.

The term canola is a registered trademark of the Canola Council of Canada (http://www.canola-council.org/) and refers to cultivars of oilseed rapeseed that produce seed oils with less than 2% erucic acid and meals with less than 30 μmol of aliphatic glucosinolates per gram (Raymer, 2002). Current canola varieties are essentially free of erucic acid and glucosinolates. Canola and rapeseed are members of the mustard family and are closely related to turnip (*Brassica rapa* L.), cabbage (*Brassica nigra* L.), cauliflower (*Brassica oleracea* var. *Botrytis* L.), broccoli (*Brassica oleracea* L).

We have previously reported that canola can be harvested at pre-flowering stage for human consumption. These results (Bhardwaj *et al*., 2003) indicated potential yield of approximately 11 t ha^{-1} of fresh greens and approximately 1 t ha^{-1} of dry matter. Canola greens contained 3.4% oil and 30.6% protein on dry weight basis (Bhardwaj *et al*., 2003). Canola greens contained 0.52, 4.14, 0.35, 1.59 and 0.20% (dry weight basis) of phosphorus, potassium, magnesium, calcium and sodium, respectively. Canola greens also contained 0.94, 2.02, 5.47, 14.65, 28.61, 0.74 and 31.92 (mg/100 g dry weight basis) of sulfur, boron, zinc, manganese, iron,copper and aluminum, respectively (Bhardwaj *et al*., 2003). The

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oil in canola greens contained 18.79, 81.14, 15.36 and 65.78% saturated, unsaturated, Mono-Unsaturated Fatty Acids (MUFA) and Poly-Unsaturated Fatty Acids (PUFA), respectively (Bhardwaj *et al*., 2003).

In many parts of the world, pre-flowering plant tissue from mustard (*Brassica juncea* L.) are harvested for the human consumption. It is well known that mustard plants regrow after these harvests to facilitate the seed harvests. Such information about canola (*Brassica napus* L. and *Brassica rapa* L.), a relative of mustard plant, is not available. The objectives of this research were to determine the potential of canola harvested for greens for seed production and to characterize the quality of seed produced by regrowth. The impetus for this study was provided by the observation that if pre-flowering canola could be harvested for human consumption and/or as forage and the regrowth could be harvested for seed, it would help in adoption of this new crop.

MATERIALS AND METHODS

We conducted two experiments to determine contents of oil and fatty acids in canola regrowth.

In the first experiment, seed of Virginia, a locally adapted cultivar developed by Virginia State University, were planted in 96 three-row field plots. The rows in these plots were 30 cm apart with each three row plot separated by 0.6 m. The sowing was done by hand on October 2, 2001 using approximately 5 kg seed ha^{-1}. Upon stand establishment, each plot received 100 kg ha^{-1} each of N, P and K. This experiment was conducted at Randolph Farm of Virginia State University located in Petersburg, Virginia (37°-15' N and 077°-30.8' W) during 2001-02 season on Abel sandy loam soil.

Before, the onset of flowering, the plots were randomly assigned to two treatments: cut vs. uncut. The total foliage in plots designated cut was harvested by hand, about 5 cm above the ground level (Bhardwaj *et al*., 2003), before appearance of yellow petals whereas the companion 48 plots (uncut plots) were not harvested. The cut plots were fertilized with a factorial combination of four rates each of P and K fertilizers (0, 50, 100, 200 kg ha^{-1}), RCBD design with three replications, to determine if varying the P and K rates had any effect on canola regrowth. All cut plots received 100 kg N ha^{-1}. In the second experiment, three rates each of N, P and K were applied to cut plots of Virginia canola cultivar in a RCBD design with two replications. This experiment was conducted at two locations in Virginia (Petersburg with Abel sandy loam soil and Suffolk (36.69° N and 76.66° W) with Rains fine sandy loam soil). Before imposition of the cut treatment (Removal of total foliage about 0.05 m above the ground level before appearance of yellow petals) all 108 plots received 100 kg ha^{-1} each of N, P and K.

Upon maturity, all cut and uncut plots were harvested by hand, threshed with a stationary thresher, to record seed yield. The seed produced in both cut and uncut plots in the first experiment and all cut plots in the second experiments were analyzed to determine the contents of oil and fatty acids. The oil was extracted from canola seeds by homogenization with hexane/isopropanol (3:2, v/v) (St. John and Bell, 1989). The Fatty Acid Methyl Esters (FAME) of canola oil were prepared as described by Dahmer *et al*. (1989). Analysis of the FAME were performed in a Varian model Vista 6000 Gas Chromatograph equipped with a fused silica capillary column (SP-Wax10, 25 m×0.25 mm i.d.), a flame ionization detector and a Spectra Physics model 4290 integrator. Carrier gas was He at a column flow rate 0.8 mL min^{-1} with a split ratio of 1:80. Oven, Injector and detector temperatures were maintained at 210, 240 and 260°C, respectively. Peaks were identified by comparison to retention of FAME standards and quantified by the aid of 17:0 as an internal standard.

Data were analyzed by procedures in version 8 of SAS statistical software (www.sas.com). In order to compare cut and uncut treatments for various traits, data were combined over different rates of P and K fertilizers. In order to determine effects of P and K fertilizer rates on various traits in cut plots, only the data from cut plots were used. Fisher's protected least significant difference at 5% level of significance was used for mean separation.

RESULTS

Significant differences existed between the seeds produced on regrowth (cut plots) and the uncut plots for seed yield, oil content and a majority of fatty acids (Table 1). The mean seed yield from 48 cut plots was 67% of that from 48 uncut plots (1349 vs. 2020 kg ha^{-1}). The oil content in regrowth cut plots was significantly lower than that in uncut plots (34.7 vs. 37.1%).

With regards to the C16:1, C18:0, C18:3, C20:0, C20:1 and C24:0 fatty acids, the seeds produced on regrowth had significantly lower contents as compared to the uncut plots whereas, the content of C18:1 fatty acids was significantly increased by the cut treatment.

The content of total unsaturated fatty acids in the seed produced on the regrowth (89.4%) was significantly greater than that in the seed produced on uncut plants (88.5%) whereas the content of total saturated fatty acids in the seed produced on regrowth (10.6%) was significantly lower than that produced on uncut plants

Table 1: Seed yield, and contents of oil and fatty acids in seeds produced on uncut and regrowth canola plants

Parameters	Regrowth[1]	Uncut[2]
Seed yield (kg ha^{-1})	1349.00	2019.97**
Oil content (%)	34.69	37.07**
Fatty acids in the oil (%)		
16:00	7.42	7.53
16:01	0.72	0.90**
18:00	2.05	2.36**
18:01	61.96	59.91**
18:02	18.66	18.00
18:03	6.39	8.09**
20:00	0.67	0.87**
20:01	1.39	1.64**
22:00	0.32	0.36
22:01	0.29	0.34
24:00:00	0.14	0.41**
Unsaturated	89.40	88.52*
Saturated	10.60	11.48*
Mono-unsaturated	64.36	62.54**
Poly-unsaturated	25.04	25.98

[1]The plants in this treatment were cut about 5 cm above ground just before flowering and the seed were harvested from regrowth. [2]The plants in this treatment were never cut and the seed were harvested from uncut plots. *, **Significant differences between means at 0.05 and 0.01, respectively

Table 2: Effects of P fertilizers on contents of oil and fatty acids in canola seed produced on regrowth[1]

	P (kg ha^{-1})				
Parameters	0	50	100	200	LSD (5%)
Seed yield (kg ha^{-1})	1521.0	1245.0	1298.0	1332.0	ns
Oil content (%)	34.8	34.0	35.3	34.6	ns
Fatty Acids in the oil (%)					
16:00	7.5	7.5	7.1	7.6	ns
16:01	0.7	0.7	0.6	0.9	0.2
18:00	2.0	2.0	1.9	2.3	ns
18:01	64.1	61.2	60.7	61.8	1.7
18:02	16.8	19.4	20.1	18.3	1.4
18:03	5.7	6.7	7.1	6.1	1.0
20:00	0.7	0.6	0.6	0.8	ns
20:01	1.5	1.3	1.3	1.4	ns
22:00	0.4	0.3	0.3	0.3	0.1
22:01	0.3	0.2	0.3	0.3	ns
24:00:00	0.2	0.1	0.1	0.2	0.1
Unsaturated	89.1	89.5	90.1	88.9	ns
Saturated	10.9	10.5	9.9	11.1	ns
Mono-unsaturated	66.6	63.4	62.9	64.5	1.7
Poly-unsaturated	22.5	26.1	27.2	24.4	2.3

[1]The plants in this treatment were cut about 5 cm above ground just before flowering and the seed were harvested from regrowth. ns: The differences among P rates were not significant. The effects of K rates on all traits under study were not significant

(11.5%). Similarly, the content of mono-unsaturated fatty acids was significantly higher in the seed produced on regrowth (64.3%) as compared to the seed produced on uncut plants (62.5%).

The effects P fertilizer rates on contents of oil and fatty acids were significant for C16:1, C18:1, C18:2, C18:3, C22:0, C24:0, mono-saturated and poly-unsaturated fatty acids (Table 2). Increasing rate of P increased contents of C16:1, C18:2, C18:3 and PUFA fatty acids whereas contents of C18:0, C22:0 and MUFA fatty acids was decreased. The effects of K rates on oil and fatty acids in cut plots were not significant.

Application of three rates each of N, P and K to cut plots (second experiment) at two locations in Virginia indicated that effects of N rates on contents of oil and fatty acids were not significant (data not presented). Effects of P rates were significant only for C18:0 and C18:3 fatty acids when significant increase in content of C18:0 resulted from increase in fertilizer rate from 0 to 50 kg ha^{-1} and significant increase in content of C18:3 resulted from increase in fertilizer rate from 0 and 50 to 100 kg ha^{-1}. Increasing the rate of K fertilizer from 0 to 50 kg ha^{-1} resulted in significant increase in content of C 22:0 whereas increase in K fertilizer rate from 0 and 50 to 100 kg ha^{-1} resulted in significant increase in the content of C18:2 fatty acid (data not presented).

DISCUSSION

Results of these studies were encouraging because it was indicated that canola could be used as a dual purpose crop. We believe that this is the first report where quality of seed produced by canola regrowth has been characterized. The seed yield produced by regrowth was 67% of the yield produced by uncut plots. However, seed produced by regrowth had significantly different fatty acid contents. The content of erucic acid (C22:1 fatty acid) was not affected by the cut treatment indicating that harvesting of foliage did not negatively affect the oil quality of canola seed. Any increase in the erucic acid content above 2% can render the canola oil unsuitable for human consumption (Raymer, 2002). A significant reduction in the content of linolenic fatty acid (C18:3) indicates that the oil from seeds produced on regrowth might be more suitable for use as specialty canola oil which normally contains lower amounts of this fatty acid (Raymer, 2002).

The content of total unsaturated and Mono-Unsaturated Fatty acids (MUFA) was improved by the cut treatment. These observations indicate that oil from regrowth plots may be healthier since it contained less saturated and more unsaturated fatty acids.

It is observed that increased P fertilizer rate increased the content of C18:3 (linolenic) fatty acid indicating that a consideration may need to be given to application of higher rates of P fertilizer for obtaining comparatively healthier oil since higher rate of C18:3 are considered desirable in human diet. However, these are preliminary data and need to be confirmed with additional experiments. However, the results suggest that residual N fertilizer from application before removal of canola foliage may be adequate but P and K fertilizers may need to be considered for cut plots.

Earlier research had also indicated that application of 100 kg ha^{-1} of N to cut and uncut canola plots is adequate. However, such information about the P and K fertilizer usage was not available. This study indicated that additional application of P and K fertilizers did not affect seed yield and oil quality of regrowth canola. This is positive observation indicating a farmer could put canola to multiple uses without any need for additional input. An unresolved issue is the cost of harvesting canola foliage. However, a preliminary survey of extension personnel in Virginia has indicated that most Virginia farmers own a corn siler-chopper that could be used to green chop canola.

The results of current study along with earlier finding that pre-flowering canola foliage can be harvested to yield 11 Mg ha^{-1} of fresh greens and 1 Mg ha^{-1} of dry matter could be of great potential for small farmers. Harvesting of canola at flowering for use as food and/or feed in combination with enhanced quality of oil from seed produced by regrowth can provide an opportunity for small farmers in Virginia and other states to increase their farm incomes. Any small farmer with some livestock can harvest the canola before flowering to use it as a feed and then harvest the seed from regrowth to market it as an oilseed. Obviously, use of canola as a multiple-use crop can be extremely helpful until a stable and consistent local market develops in the Southern United States for oilseed canola.

CONCLUSION

Present results showed that the potential of canola as a dual purpose crop to enhance the income of small farmers. It could be harvested before flowering as a value-added feed and food product and the regrowth could be harvested for seeds to provide edible oil.

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