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Influence of nitrogen and sulfur fertilization on quality of canola (*Brassica napus* L.) under rainfed conditions*

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Abstract: Field experiments were conducted at Cereal Crops Research Institute, Pirsabak, Nowshera, Pakistan, during winter 2003~2004 and 2004~2005 to evaluate the effect of nitrogen and sulfur levels and methods of nitrogen application on canola (*Brassica napus* L. cv. Bulbul-98) under rainfed conditions. Four levels of S (0, 10, 20, and 30 kg/ha) and three levels of N (40, 60, and 80 kg/ha) and a control treatment with both nutrients at zero level were included in the experiments. Sulfur levels were applied at sowing while N levels were applied by three methods (100% soil application, 90% soil+10% foliar application, and 80% soil +20% foliar application). The experiments were laid out in randomized complete block (RCB) design having four replications. Oil content increased significantly up to 20 kg S/ha but further increase in S level did not enhance oil content. Glucosinolate content increased from 13.6 to 24.6 μmol/g as S rate was increased from 0 to 30 kg/ha. Protein content increased from 22.4% to 23.2% as S rate was increased from 0 to 20 kg/ha. Oil content responded negatively to the increasing N levels. The highest N level resulted in the highest values for protein (23.5%) and glucosinolate (19.9 μmol/g) contents. Methods of N application had no significant impact on any parameters under study.

Key words: Canola, Nitrogen (N), Sulfur (S), Methods of N application, Quality
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INTRODUCTION

Pakistan has been facing a chronic shortage of edible oil; a large quantity of edible oil is imported annually from other countries to fill the gap between local production and consumption. In 2003~2004, the total consumption was estimated as 2.199 million tons and the local production was sufficient to meet merely 29% of the consumption; while the remaining 71% was met through imports (GOP, 2004).

Rapeseed is an annual rabi crop, 50~200 cm tall and branched with a tap root system and many lateral roots concentrated in the shallow sub-surface soil. In Pakistan, more than 50% of the rapeseed crop is grown in the eastern province Punjab and more than 90% of it is barani species. However, rapeseed *Brassica napus* is becoming popular in farming communities of Pakistan because of its high yields and high quality oil for human consumption. The young leaves of rape and mustard are used as vegetables and fodder, whereas the oil is used in cooking, in making pickles, and in the lubricant industry (Hatam and Abbasi, 1994).

The rapeseed and mustard are the second source of edible oil after cotton seed contributing towards the national production of edible oil, but its oil is of low quality due to the presence of high concentration of erucic acid and glucosinolate. Erucic acid and glucosinolate are considered toxic for both human and animals' health in addition to its bitter taste (Muhammad *et al*., 1991). Safe limits for these compounds have been described as less than 2% erucic acid in oil and less than 30 μmol/g of

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glucosinolate in oil free meal (Grombacher and Nelson, 1992). Canola has the lowest saturated fat content among the vegetable oils and thus has an increasing demand for the diet-conscious consumers (Grombacher and Nelson, 1992). The tender leaves of these cultivars serve as vegetable and the seed as a source of cooking oil. The residue left after oil extraction being rich in protein is used in livestock feed (Khalil *et al*., 1995).

Canola is adapted to a wide range of environmental conditions. The crop can be cultivated above the polar circles where there is 24 h of daylight during the summer months as well as in Pakistan where day length is less than 10 h during the winter. Rapeseed grows best under relatively cool temperatures up to flowering. After flowering it can tolerate high temperature but heat and drought stress may result in a reduction of seed size, crop yield and oil contents (Rehman *et al*., 1987). Current management by the rainfed farmers in Pakistan is at very basic levels of technology (Khan *et al*., 2004), so the opportunities for increased productivity with improved management practices are substantial.

Compared to cereals, winter oilseed rape requires a higher amount of nutrients and available nitrogen (N) (Rathke *et al*., 2005; Hocking *et al*., 1997), which is worth to be considered in relation to its role in affecting quality and quantity. Compared to cereals, it has a higher critical N demand (Colnenne *et al*., 1998). Choosing the correct rate and timing of N fertilizer application is one of the most important aspects of successful oilseed rape production (Holmes, 1980). Canola yield and nutrient uptake are highly dependent on N fertility and peak seed yields occur with 120 to 180 kg N/ha (Jackson, 2000). Optimizing the yield of oilseed rape involves balancing the synthesis of oil and crude protein in the seeds. Many researchers indicated that oil content of oilseed rape declined with increasing rate of N fertilizer that had positive effect on crude protein (Rathke *et al*., 2005), but Hocking *et al*.(1997) and Brennan *et al*.(2000) are of the opinion that high N rate did not always affect the oil content.

In the earlier research, canola or rapeseed has been shown to respond markedly to sulfur (S) fertilization on S-deficient soils (Janzen and Bettany, 1984; Nuttall *et al*., 1987). Applying about 20 kg S/ha can satisfy canola's high S requirement (Jackson, 2000). Oil, protein and glucosinolate concentrations in seed increase with S fertilization (Malhi *et al*., 2007; Haneklaus *et al*., 1999). S fertilizer application also improves N-use efficiency and thereby maintains a sufficient oil level and fatty acid quality (Fismes *et al*., 2000). But inconsistent responses of different *Brassica* species/cultivars to S fertilization under controlled conditions and in field studies have also been reported (Wetter *et al*., 1970; Aulakh *et al*., 1980; Nuttall *et al*., 1987; Ahmad and Abdin, 2000).

Keeping in view the importance of N and S in affecting quality parameters of canola, the present experiments were conducted to study the effects of N and S levels and methods of N application on quality parameters of canola under rainfed conditions.

MATERIALS AND METHODS

Experimental site

Field experiments were conducted at Cereal Crops Research Institute Pirsabak, Nowshera, Pakistan, during winter 2003~2004 and 2004~2005. Nowshera is located about 1600 km north of Indian Ocean at 34° N latitude, 72° E longitude and an altitude of 288 m above sea level. The soil of the experimental field was sandy loam, moderately calcareous, low in N (0.014%), low in organic matter (0.31%), low in available S (8.27 mg/kg) and alkaline in reaction having a pH of 7.7. Temperature and rainfall during the crop growing season have been shown in Table 1. Weather data were collected from the meteorological station located near the experimental site.

Experimental procedure

Seed of improved canola cultivar Bulbul-98 was sown on Oct. 18, 2003 and Oct. 19, 2004. The seed was sown with a uniform seed rate of 5 kg/ha in all plots with the help of hand hoe in straight rows. The experiments were laid out in randomized complete block design having four replications. The size of sub plot was 5 m×3 m. In each sub plot there were 6 rows 5 m long and 50 cm apart. Fertilizer basic dose of P and K at the rate of 60 and 60 kg/ha was applied in the forms of triple super phosphate and murate of potash respectively prior to sowing. Four levels of S, 0 (S1), 10 (S2), 20 (S3) and 30 (S4) kg/ha and three levels of N, 40 (N1), 60 (N2) and 80 (N3) kg/ha were included

Table 1 Average air temperature and total rainfall at the experimental site during the crop growing season 2003~2004 and 2004~2005

Month	T_{min} (°C)	T_{max} (°C)	T_{ave} (°C)	Total rainfall (mm)	Ten-year average rainfall (mm)*
October	15.4 (14.7)	30.9 (28.0)	23.2 (21.7)	14.0 (106.2)	32.1
November	17.7 (9.0)	24.4 (26.8)	21.1 (17.9)	41.0 (19.7)	12.8
December	7.0 (7.2)	19.7 (20.1)	13.4 (13.6)	43.0 (104.1)	23.0
January	6.2 (3.8)	16.8 (17.0)	11.5 (10.8)	70.5 (36.0)	50.3
February	6.8 (6.8)	21.6 (16.3)	14.2 (11.5)	58.5 (114.0)	63.6
March	13.4 (12.4)	30.1 (24.9)	21.8 (18.7)	0.0 (127.7)	52.7
April	18.0 (14.9)	33.2 (30.9)	25.6 (22.9)	65.8 (17.5)	24.5
Total rainfall during the growing season				292.8 (525.2)	259.0

Without parenthesis: For crop season 2003~2004; With parenthesis: For crop season 2004~2005. T_{min}: Minimal temperature; T_{max}: Maximal temperature; T_{ave}: Average temperature. * Ten years: 1995~1996 to 2004~2005

in the experiments. One treatment of both N and S at zero level was kept as control. Methods of N applications consisted of 100%, 90% and 80% placement at sowing along with 0%, 10% and 20% foliar spray at stem elongation stage, respectively. N was applied in the form of urea while S was applied in the form of ammonium sulfate. The amount of ammonium sulfate was calculated to supply the required level of S. The quantity of N supplied by the calculated amount of ammonium sulfate was calculated, and the remaining N was then supplied by urea. Combinations of N and S levels and methods of application of N were allotted at random to plots in each replication. The N levels were applied by placement at the time of sowing and foliar spray at stem elongation stage. By placement at the time of sowing 100%, 90% and 80% of all the levels were applied, while remaining was applied in the form of foliar spray. For foliar application, first water was sprayed on a plot to find out the volume of water required for a plot. The calculated amount of urea was dissolved in the required volume of water and then the diluted solution was sprayed on the crop through hand operated sprayer. Pure water was sprayed on those plots, which did not receive foliar application of N. After the completion of germination, seedlings were hand thinned to maintain a uniform plant to plant distance of 5 cm. Weeds were controlled manually. All cultural practices were applied uniformly to all the plots. The experiments were harvested in April during both years. Data were recorded on oil content (%), glucosinolate contents (μmol/g) and protein content (%).

Procedure for data recording

Random seed samples from each plot were collected and analyzed for oil, glucosinolate, and proteins by FOSS Routine Near Measurement System (35RP-3752F) TR-3657-C Model 6500 (Maryland, USA), at oilseed laboratory, Nuclear Institute for Food and Agriculture, Peshawar, Pakistan. Near infrared reflectance (NIR) spectroscopy is a rapid, non-destructive whole seed scanning technique, which does not require any sample preparation or chemicals (Daun *et al*., 1994).

Statistical analysis

Data recorded were analyzed statistically, using analysis of variance (ANOVA) techniques appropriate for randomized complete block design. For analysis excel worksheet was programmed. Main and interaction effects were compared using least significant difference (LSD) test at 5% level of probability, when the *F*-values were significant. One single degree of freedom contrast was used to compare control with the rest of main plot treatments (Steel and Torrie, 1980).

RESULTS

Oil content

Data regarding oil content of canola as affected by N and S are shown in Table 2. The N and S levels significantly affected oil content of canola. Interaction between the N and S levels was significant for percent oil in canola. No significant influence of N application methods (M) was observed on oil contents. Similarly interactions of N×M, S×M, and N×S×M were also not significant. Comparison of the control vs fertilized plots means indicated that control plots produced significantly higher oil contents (43.5%) than the average oil contents (42.4%) produced by the plots which received N and S doses. Increasing N levels had a depressing effect on the oil contents of canola. Higher oil contents were produced by the lower N rate of 40 kg/ha while lower oil contents were recorded by the highest dose of 80 kg N/ha. Contrary to the response of oil content to N doses, S had a positive impact on oil contents of canola. The lowest oil contents (41.9%) were found in those plots, where no S was applied. Oil contents enhanced to 42.8% with the application of 20 kg S/ha but further increase to 30 kg S/ha had no significant influence on oil contents. The significant N×S interaction revealed that oil content was higher at 10, 20 and 30 kg S/ha in combination with the lowest rate of 40 kg N/ha. However, further increase in N level did not increase oil content in canola with any level of S. Minimum oil content was recorded at high dose of N without S application. Mean comparison of the two years data revealed that higher oil contents (43.4%) were recorded in 2004~2005 as compared to 2003~2004 (42.5%).

Glucosinolate content

Data regarding glucosinolate content of canola as affected by N and S are shown in Table 2. Perusal of the data revealed that different levels of N and S significantly affected glucosinolate content of canola. The N application methods had no significant impact on glucosinolate contents. A significant N×S interaction was also detected for glucosinolate contents. The interactions of N×M, S×M and N×S×M were not significant for glucosinolate contents. Significantly higher value of the glucosinolate contents (19.0 μmol/g) were found in the plots receiving NS nutrition as compared to control plots (13.4 μmol/g). Glucosinolate contents increased from 18.0 to 19.9 μmol/g as N rate increased from 40 to 80 kg N/ha. Similarly, glucosinolate contents increased from 13.6 to 24.6 μmol/g when S rate increased from 0 to 30 kg S/ha indicating that the increase due to S rate was much higher than the increase due to N rates. Significant N×S interaction indicated that glucosinolate content increased with increase in both S and N and higher glucosinolate content was recorded in the plots that received 80 kg N/ha and 30 kg S/ha. Years as a source of variation revealed that higher glucosinolate contents of 16.5 μmol/g were recorded in 2003~2004 as compared to 15.8 μmol/g in 2004~2005.

Protein content

Data regarding protein content of canola as affected by N and S are shown in Table 2. The effects of N and S on protein content of canola were significant while the effect of methods of N application was not significant. All the interactions among the three factors were not significant. Higher value of the protein content (22.9%) was noted in the plots supplied with NS nutrients as compared to

Table 2 Oil content (%), glucosinolate content (μmol/g) and protein content (%) of canola as affected by S and N levels and its methods of application under rainfed conditions

	Oil content (%)	Glucosinolate content (μmol/g)	Protein content (%)
N levels (kg/ha)			
40	43.2 a	18.0 c	22.3 c
60	42.6 b	19.0 b	23.0 b
80	41.6 c	19.9 a	23.5 a
S levels (kg/ha)			
0	41.9 c	13.6 d	22.4 c
10	42.5 b	16.9 c	22.8 b
20	42.8 a	20.9 b	23.2 a
30	42.6 b	24.6 a	23.3 a
Methods			
M1	42.5	18.8	22.9
M2	42.5	19.0	23.0
M3	42.4	19.2	23.0
Year			
2003~2004	42.5 b	16.5 a	21.9 a
2004~2005	43.4 a	15.8 b	21.7 b
Control vs rest			
Control	43.5 b	13.4 b	20.6 b
Rest	42.4 a	19.0 a	22.9 a
Interactions			
S×N	*	*	ns
S×M	ns	ns	ns
N×M	ns	ns	ns
S×N×M	ns	ns	ns

Means of the same category followed by different letters are significantly different from one another using LSD test at 5% level of probability. N: Nitrogen; S: Sulfur; M: Methods; * Significant at 5% level of probability; ns: Non-significant control plots (20.6%). Mean values for N rates revealed that seed protein contents enhanced progressively with increase in N rates and the highest protein content of 23.5% was found at the maximum level of 80 kg N/ha. Similarly, seed protein content also had a positive response to the increasing S levels. Higher protein contents of 23.2% and 23.3% were recorded for the plots that received 20 to 30 kg S/ha, respectively. The lowest protein content of 22.4% was noted in the plots that received no S. Higher content of seed protein (21.9%) were noted in 2003~2004 as compared to 21.7% in 2004~2005.

DISCUSSION

Oil content

Oil content did not respond to the increasing level of N. Unlikely to N, oil content increased with increase in S level from 10 to 20 kg/ha but further increase in S level did not enhance oil content. The N×S interaction values indicated that oil content was higher when N and S were applied in combination at the rate of 40 kg N/ha and 20 kg S/ha, respectively. It has been reported by several researchers that combined application of S and N enhances the oil and protein contents of the seeds of *Brassica* genotypes (McGrath and Zhao, 1996). Likewise, Zhao *et al*.(1993), Dubey *et al*.(1994), Asare and Scarisbrick (1995) and Cheema *et al*.(2001) reported low oil content with higher doses of N. However, Brennan *et al*.(2000) reported that the oil concentration of canola seed remained unaffected by N rate. Several reasons have been given by different researchers for the decrease in oil content with increasing N rates. For example, Kutcher *et al*.(2005) stated that it might be due to the dilution effect of increased seed yield with increased N fertilization and the inverse relationship of protein and oil content. Jackson (2000) believed that N delays plant maturity which results in poor seed filling and greater proportion of green seed. Holmes (1980) reported that a better supply of N increases the formation of N containing protein precursors so that protein formation competes more strongly for photosynthates; as a result less of the latter are available for fat synthesis. Likewise, Rathke *et al*.(2005) linked this fact with reduced availability of carbohydrates for oil synthesis at high N application. The decrease in oil content of canola with the increasing levels of N is consistent with other reports (Jackson, 2000; Kutcher *et al*., 2005; Rathke *et al*., 2005; Cheema *et al*., 2001). The increase in oil content with the application of S favors the views of Marschner (1986) who stated that S is involved in the synthesis of oil. These results are in agreement with Mailer (1989), Malhi and Leach (2000) and Subhani *et al*.(2003) who reported that oil content of canola increased with increase in S rate. Mean comparison of the two years data revealed that higher oil contents were recorded in 2004~2005 as compared to 2003~2004. The higher oil content in 2004~2005 can be attributed to higher and evenly distributed rainfall throughout the growing season in 2004~2005.

Glucosinolate content

Glucosinolate content responded positively to the increasing levels of both S and N. The interaction between S and N also indicated that glucosinolate contents were higher at the highest levels of S and N. Several researchers have reported that S application enhances glucosinolate content of oil seed rape (Janzen and Bettany, 1984; Schnug, 1991; Withers and O′Donnell, 1994). Similar results were reported by Chen *et al*.(2006) who suggested that accumulation of aliphatic glucosinolate and aromatic glucosinolate could be enhanced by low N and high S and restricted by high N while that of indolyl glucosinolate could be enhanced by high N and high S. The results obtained are in line with Fismes *et al*.(2000) who found that S application greatly increased the level of glucosinolate content compared with the soil receiving only N fertilization. The increase in glucosinolate contents of canola with increasing rate of S might be due to the fact that glucosinolate is an S containing compound and thus increased with adequate supply of S. Glucosinolates contents were low in those plots which did not receive S. It might be due to the S requirements of the glucosinolates molecule in those S deficient plots (Mailer, 1989). The results are in agreement with the findings of Mailer (1989), Fismes *et al*.(2000) and Wang *et al*.(1997) who reported that glucosinolate content increased significantly with S application. Similarly, Thakral *et al*.(1996) reported that glucosinolate content increased with increasing rate of N. Likewise, Fismes *et al*.(2000) entailed that a better control of glucosinolate content relies on the balanced fertilization of both N and S.

Protein content

Protein content enhanced with increasing levels of both S and N. The increase in seed protein content of canola with the application of N and S could be due to the fact that N is an integral part of protein and the protein of rapeseed contains relatively large quantities of the S containing amino acids like methionine and cystine (Gardner *et al*., 1985). The high protein content at high level of N may be due to the negative correlation between oil content and protein content (Hao *et al*., 2004) as oil content decreased with increase in N level in this study. The physiological reason for the negative correlation may be that the carbohydrate content of protein is lower than that of oils (Lambers and Poorter, 1992); increased N supply intensifies the synthesis of protein at the expense of fatty acid synthesis and thus reducing the oil content of the seed (Rathke *et al*., 2005). The increase in protein content with the increase in N rate confirmed the findings of Dubey *et al*.(1994) and Kutcher *et al*.(2005) who found that protein contents of canola increased significantly with the increasing N rates. The results obtained are in agreement with the earlier findings of Wang *et al*.(1997) and Malhi and Leach (2000) who stated that applied S increased protein content of canola.

CONCLUSION

The findings show that oil and protein contents of canola seed responded positively to the increasing level of S but it also enhanced gulcosinolate contents, which was not desirable. Increasing levels of N progressively enhanced protein and glucosinolate contents up to the highest level of 80 kg N/ha. Unlikely, oil content decreased with increasing N levels. No significant impact of N application methods was found.

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