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Effects of Sulphur on Seed Yield, Oil, Protein and Glucosinolates of Canola Cultivars

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ABSTRACT

Sulphur is the fourth major nutrient in crop production. Most crops require as much sulphur as phosphorus. Canola cultivars has recent introduction in Pakistan's agriculture so information related to sulphur effects on seed yield and seed composition are scanty. A two year study (2003 - 04 & 2004 - 05) was conducted to document the effects of sulphur application on seed yield, oil, protein and glucosinolates of canola cultivars. Two canola cultivars and four sulphur levels were arranged in randomized complete design with split plot arrangement. Cultivars exhibited statistically significant variations for protein but non-significant differences for seed yield, oil and glucosinolates. Similarly, sulphur effects on seed yield, oil, protein and glucosinolates were neither significant nor consistent. However, interactive effects were observed to be significant. Seed yield, protein and glucosinolates increased during second year as compared to those observed during first year, whereas oil content exhibited an opposite trend and decreased during second year as compared to first year. Inverse relationship was observed between oil and protein during both the years of experiments. However, glucosinolates exhibited linear relationship to sulphur levels but did not show any significant relationship with seed yield.

Key Words: Sulphur; Seed yield; Oil; Proteins; Glucosinolates

INTRODUCTION

Edible oil is one of the basic requirements of our daily diet. Pakistan is encountered with acute deficiency of edible oil because of its increased consumption. At present the annual edible oil requirement of the country is about 2 million tones of which only 30% is met by local production and rest 70% being imported (GOP, 2006).

Brassica crops and oilseed rape in particular, are a means of producing high yields of good quality oil for human consumption. Nutritionally, oilseed rape and *Brassica* species in general require sulphur during their growth (Zhao *et al*., 1993), for the synthesis of both protein and naturally occurring glucosinolates. Both sulphur uptake and sulphur translocation in oilseed rape varieties vary as a function of growth stage and plant part. However, as compared with single low varieties double low varieties contain lower seed Sulphur concentration and higher pod sulphur concentrations. Oilseed rape is particularly sensitive to sulphur deficiency. Nitrogen and sulphur are both involved in plant protein synthesis. The shortage in sulphur supply for crops decreases the N-use efficiency of fertilizers (Ceccoti, 1996). Consequently, the poor efficiency of N caused by insufficient S needed to convert N into biomass production may increase N losses from cultivated soils (Schnug *et al*., 1993). Canola has high requirements for Sulphur (Grant & Bailey, 1993), due to a combination of high protein content with high proportions of cysteine and methionine (Clandinin, 1981).

According to Andersen *et al*. (1996) seeds may be regarded as consisting of nitrogen-free structural material, stored proteins and stored oil. The proportion of structural material is expected to decrease with increasing seed weight, while oil and protein may compete for remaining space in seeds. Canola seeds contain about 39 to 48% oil, which is mainly influenced by moisture and temperature during reproductive stages (Si *et al*., 2003). Cooler and moist spring has been concluded to be conducive for higher oil accumulation. Increased oil would lower protein accumulation (Pritchard *et al*., 2000). Higher oil accumulation would be beneficial for oil extracting industry, however low protein will decrease the quality of feed to be used for livestock.

Sulphur is the fourth major nutrient in crop production. Most crops require as much sulphur as phosphorus. The nitrogen and sulphur requirements of crops are closely related, because both nutrients are required for protein synthesis. Sulphur is involved in the synthesis of chlorophyll and is also required for the synthesis of oil (Marschner, 1986).

Sulphur also plays an important role in the chemical composition of seed. Sulphur increases the percentage of oil content of the seed (Chaudhry *et al*., 1992) and glucosinolate content (Marschner, 1986). Glucosinolates are a group of secondary metabolites containing β-thioglucose, a sulphonated oxime moiety and a side chain, their concentration is closely related to the S supply, what is not surprising, since each glucosinolate molecule contains two or three S atoms (Zhao *et al*., 1997). Sulphur supply has not only an impact on the total concentration of glucosinolates in the rapeseeds, but also on the relative proportions on the individual glucosinolates. Due to interactions between S and

N, the influence of the S nutritional status of rape on glucosinolate synthesis should always be considered along with the N supply.

Sulphur is required in smaller quantities by most of the cereal crops so it has been completely neglected for minor crops like canola. Canola cultivars were introduced in Pakistan during last decade so not much research work has been done particular on quality aspects of the produce. Therefore, keeping in view the importance of oilseed in Pakistan's agriculture and sulphur for oilseed rape, present study was under taken to record the sulphur effects on yield and seed composition of two canola cultivars grown under rain fed areas. Consequently, this study was based on the hypothesis, "sulfur application may enhances the yield and alter the seed composition of Canola".

MATERIALS AND METHODS

The experiments to evaluate sulphur effects on seed yield and seed composition of canola cultivars were conducted at the University of Arid Agriculture, Rawalpindi, Pakistan during 2003 - 04 and 2004 - 05. The soil of particular site contained 15% clay, 45% silt, 40% sand, had bulk density of 1.45 Mg cm^{-3}, pH 7.7, EC 0.25 d S m^{-1}, 0.66% organic matter, 3.84 µg g^{-1} N, 6.5 µg g^{-1} P, 130 ppm K and 7.6 mg kg^{-1} SO_4^- S.

The experiments were laid out in randomized complete block design with split plot arrangement. The cultivars were kept in main plots and sulphur levels in sub-plots replicated four times in net plot size of 5 m x 2.7 m. Seeds of cultivars Shiralee (C1) and Con-11(C2) were drilled with hand drill using seed rate of 5 kg ha^{-1}. There were six rows in each plot 45 cm apart. The particular fields were summer fallow. Soil was prepared by plowing the field with tractor mounted cultivator four times and planked with last ploughing. Fertilizers N and P were incorporated @ 80 - 40 kg ha^{-1} in the soil with last ploughing in the form of urea and DAP. Elemental sulphur (S1 = Control, S2 = 15 kg ha^{-1}, S3 = 20 kg ha^{-1} & S4 = 25 kg ha^{-1}) weighed for each plot was spread immediately after sowing and mixed with soil manually. Weeds were kept under control by manual weeding when needed. At maturity central two rows were harvested manually on 01 - 04 - 2004 and 13 - 04 - 2005, of first and second year experiment, respectively. Harvested plants were tied in small bundles and kept up-right with wall for sun drying for five days. After drying plants were thrashed manually. Seeds were cleaned with small blower. Seed yield was recorded on plot basis and then converted into hectare basis. Oil, protein and glucosinolates were determined with near-infrared reflectance spectroscopy (NIR) systems, Foss 6500, USA. The recorded data were subjected to statistical analysis appropriate to Randomized Complete Block Design by using M. Stat.C (Freed & Eisensmith, 1986). Means were compared for significance at 5% probability level using LSD (Steel & Torrie, 1980).

RESULTS

Cultivars exhibited non-significant differences for seed yield. However, cultivar x year interaction showed significant differences. Comparatively higher seed yield was observed during second year as compared to first year. Seed yield of the both cultivars increased during second year however, Con-II showed increase of 10% in comparison to Shiralee, which depicted only 3% increase. Sulphur levels affected seed yield non-significantly. However, sulphur x year interaction exhibited significant differences. During second year, comparatively more seed yield was observed as compared to first year. Cultivars x sulphur averaged over years exhibited statistically significant differences. C1 x S2 produced the highest seed yield of 2005.7 kg ha^{-1} followed by C2 x S4. Rest of interactions, were observed to be non-significant. During season I, C1x S2 produced the highest (1819.8 kg ha^{-1}) seed yield followed by C1 x S1 and C2 x S4. During season II, again C1 x S2 gave the highest seed yield (2191.8 kg ha^{-1}), however, interactions depicted varying degree of differences. All interaction exhibited increase during second year as compared to first year (Table I). Comparison of year exhibited significant differences.

Brassica cultivars did not show statistical differences for oil content. Mean oil content remained statistically same during both the years of experiments. However, a small reduction of oil content in both the cultivars was observed during second year. Years effect on oil content was found to be statistically non-significant. The mean values for varying levels of sulphur during both the years exhibited significant differences. The S4 (25 kg ha^{-1}) accumulated maximum oil content (48.9%) during 2003 - 04, whereas the maximum oil accumulation (48.2%) during 2004 - 05 was recorded in S3 (20 kg ha^{-1}). Response of oil content to sulphur levels was not consistent during both the cropping seasons. Oil content of S1 (control) and S3 (20 kg ha^{-1}) exhibited a small increase, while S2 (15 kg ha^{-1}) and S4 (25 kg ha^{-1}) showed a reduction of 1.7 and 2%, respectively during second year in comparison to first year (Table II).

Cultivar x sulphur interactive effects showed significant response. The maximum oil accumulation was recorded in treatment C2 x S4. Interactive effects cultivar x sulphur x year were found to be statistically significant. During 2003 - 04, C1 x S4 accumulated the maximum oil content (48.9%). However, C1 x S3 accumulated the maximum oil content (48.6%) during 2004 - 05. Oil content recorded in both of the cultivars (Shiralee & Con-II) affected by sulphur levels in present study revealed minor differences between cultivar and sulphur levels (Table II).

Table I. Effect of sulphur seed yield (kg/ha) of two canola cultivars

Sulphur levels	Cultivars						Cultivars Mean
	Shiralee (C_I)			Con-II (C_{II})			
	Years			Years			
	2003-4	2004-5	Mean	2003-4	2004-5	Mean	(C_I): 1879
S1(control)	190	19687	1879	1598	1977	1787	(C_{II}): 1832
S2 (15 kg)	1819	2191	2005	1656	2073	1865	
S3 (20 kg)	1703	1957	1830	1543	2027	1785	
S4 (25 kg)	1515	2093	1804	1755	2026	1891	
Mean	1707	2052		1638	2026		
SEM	Cultivars= 24.2 , Cultivars x Year = 34.28, Sulphur.= 47.3, Sulphur.x Year= 67.2, Cultivars x Sulphur= 67.2, Cultivars x Years x Sulphur= 95.07, Years= 24.2						

Table II. Effect of sulphur on oil contents (%) of two canola cultivars

Sulphur levels	Cultivars						Cultivars Mean
	Shiralee (C_I)			Con-II (C_{II})			
	Years			Years			
	2003-4	2004-5	Mean	2003-4	2004-5	Mean	(C_I): 47.8
S1(control)	47.3	47.8	47.5	47.8	48.2	47.9	(C_{II}): 48.1
S2 (15 kg)	47.8	47.0	47.4	48.9	47.3	48.1	
S3 (20 kg)	47.7	48.6	48.2	47.8	47.8	47.8	
S4 (25 kg)	48.9	47.6	48.3	48.7	48.1	48.4	
Mean	47.9	47.8		48.3	47.9		
SEM	Cultivars= 0.16 , Cultivars x Year = 0.22, Sulphur.= 0.25, Sulphur.x Year= 0.35, Cultivars x Sulphur= 0.35, Cultivars.x Years x Sulphur= 0.49, Years=0.16						

Table III. Effect of sulphur on protein content (%) of two canola cultivars

Sulphur levels	Cultivars						Cultivars Mean
	Shiralee (C_I)			Con-II (C_{II})			
	Years			Years			
	2003-4	2004-5	Mean	2003-4	2004-5	Mean	(C_I): 23.6
S1(control)	23.0	24.8	23.9	23.9	25.0	24.5	(C_{II}): 24.5
S2 (15 kg)	22.8	25.1	23.9	23.2	25.9	24.5	
S3 (20 kg)	23.0	24.1	23.5	23.5	25.7	24.6	
S4 (25 kg)	21.7	24.5	23.1	23.6	24.9	24.2	
Mean	22.6	24.6		23.6	25.4		
SEM	Cultivars= 0.23 , Cultivars x Years = 0.33, Sulphur= 0.25, Sulphur x Years= 0.36, Cultivars x Sulphur= 0.36, Cultivars x Years x Sulphur= 0.56, Years=0.23						

Statistically significant differences were observed for protein content of two cultivars. Con-II accumulated the highest (24.5%) protein content, whereas (23.6%) protein content was recorded in Shiralee. Years effect on protein content was found to be statistically significant (Table III). Protein accumulation increased during second year as compared to first year. Significant variations between cultivars and years could be the combined effect of genetic make of the cultivars tested and climate variations of years.

Sulphur levels did not show any impact on protein accumulation. However, sulphur impact between years was found to be significant. Protein accumulation during second year increased in all sulphur levels including control. Cultivar x sulphur interactive effects showed significant response (Table III). The highest protein content (24.6%) was recorded in treatment C2 x S3. The lowest (23.1%) protein content, were recorded in C1 x S4. The differences among mean protein accumulation due to (cultivar x sulphur x year) interactive effects were found to be statistically significant. During year 2003 - 04, C2 x S1 accumulated the highest protein content (23.9%), whereas the lowest (21.77%) protein content was recorded in C1 x S4. However, the highest protein accumulation (25.9%) was recorded in C2 x S2, while the lowest (24.1%) protein content were recorded in C1 x S3 during 2004 - 05 (Table III).

Glucosinolate of *brassica* cultivars did not show statistically significant differences for glucosinolate. Both the cultivars were at par to each other but glucosinolate accumulation was higher in second year as compared to first year. Years effect on glucosinolates were found to be statistically significant (Table IV).

Sulphur levels did not show any impact on glucosinolate accumulation and the mean values for varying levels of sulphur treatments exhibited non-significant differences. Sulphur x year effects, were found to be significant. The S3 accumulated the maximum glucosinolate (73.6 μ mol g^{-1}) during 2003 - 04, whereas the maximum (84.5 μ mol g^{-1}) glucosinolate accumulation during second season was observed with treatment S4. Glucosinolates of all sulphur levels increased during second year as compared to first year. Higher values observed during second year may be attributed to cross pollination of double low cultivars with those of traditional *brassica* cultivars grown in nearby fields.

Cultivar x sulphur interactive effects showed differential response. The C2 x S1 accumulated the maximum glucosinolate (81.9 μ mol g^{-1}). The minimum glucosinolates (68.1 μ mol g^{-1}) were observed for C1 x S1. The differences among the glucosinolate content due to cultivar x sulphur x year interaction were found to be significant. During 2003 - 04, C2 x S1 gave the maximum glucosinolate (75.3 μ mol g^{-1}), whereas the minimum glucosinolate (64.7 μ mol g^{-1}) was observed for C2 x S4. The C2 x S1 again accumulated the maximum (88.6 μ mol g^{-1}) during second year.

DISCUSSION

Sulphur response has been reported to be better on S deficient soils. Haneklaus *et al*. (1999) concluded that yield of oilseed rape increased on severe S deficient soil average by 88%. Yield on moderate S deficient sites was tendentiously higher by S fertilization but the differences were statistically non-significant. Similarly, Janzen and Bettany (1984) observed a positive response of canola seed and straw yield to increasing S rate, when adequate N was also applied, particularly on S deficient soils. In present study, response of S on seed yield has been non-significant, so results are closely related to Haneklaus *et al*. (1999).

Application or placement method would also affect the efficiency of applied S. In present study, the lack of differences for seed yield from incorporated S during first

Table IV. Effect of sulphur on glucosinolate (μ mol g^{-1}) of two canola cultivars

Sulphur levels	Cultivars						Cultivars Mean
	Shiralee (C_I)			Con-II (C_{II})			
	Years			Years			
	2003-4	2004-5	Mean	2003-4	2004-5	Mean	(C_I): 74.6
S1(control)	66.9	69.3	68.1	75.3	88.6	81.9	(C_{II}): 77.9
S2 (15 kg)	65.8	87.6	76.7	71.9	80.3	76.1	
S3 (20 kg)	72.6	79.2	75.9	74.7	86.6	80.6	
S4 (25 kg)	67.8	87.5	77.6	64.7	81.6	73.2	
Mean	68.3	80.9		71.7	84.3		
SEM	Cultivars= 1.16, Cultivars x Years = 1.64, Sulphur= 1.36, Sulphur x Years= 1.92, Cultivars x Sulphur= 1.92, Cultivars x Years x Sulphur= 2.71, Years=1.16						

season may be the result of seasonal dryness particularly during reproductive growth i.e. February-March. However, better response of the crop to applied S during second year may be related to adequate availability of moisture, relatively cooler and extended reproductive phase, which ultimately increased the final yield. It has been concluded by Grant and Bailey (1993) that spring broadcast or broadcast incorporated application of sulphate-S is readily available for plant growth under good soil moisture conditions. But under dry conditions, side banding or pre seed banding may be superior. This is a result of the bands being less subject to drying than the surface soil and application of sulphate-S in the row or in band may also provide a readily available source of S to stimulate early plant growth. So, our results are in line with above findings.

Oil synthesis appeared to be mediated via post-anthesis growth conditions. Conditions those were conducive to high assimilates supply during grain filling were positively associated resulting large seed size with high oil content. However, oil accumulation was negatively associated with mean daily temperature between anthesis and maturity. Rise of 1^{o}C temperature cause a loss of 1.2% of oil. Thus, a small negative response of oil to temperature during grain filling period is apparent (Robertson *et al.*, 2004). In present study, non-significant differences for oil content between cultivars may be related to genetic make of the cultivars tested. Small reduction may have been due to higher protein accumulation, which affected oil content. The statistically non-significant differences for oil content due to sulphur application of present study are contrary to Jan *et al.* (2002), who recorded significant increase in oil content by sulphur application (60 kg S ha^{-1}) compared to control. However, similar finding have been reported by Fismes *et al.* (2000), who recorded 1.2% lower oil in first year experiment than that of second year, which was related to drought. Our findings also revealed 1.2% reduction of oil during second year as compared to first year. However, in our study drought was not encountered, rather more rains well distributed over crop life cycle were received during second year (257.2 mm) as compared to first year (193.4 mm). An inverse relationship (Fig. 1 & 2) was observed between oil and protein during both years of experiments. Thus, findings are consistent to those of Pritchard *et al.* (2000), who recorded increase of protein with decrease of oil content and concluded that wetter and cooler spring would favor higher oil accumulation, while lower proteins. However, dry and warm spring may favor higher protein.

The seed has four components: oil, protein, water and residue. Si *et al.* (2003) concluded that in *brassica* increase of protein could be either solely at the cost of oil or solely at the expense of residue. It was further elaborated that year effects on oil and protein concentration were highly significant. The negative correlation between oil and protein concentration on seed basis has been reported mainly due to environmental factors (Mailer & Pratley, 1990). In present study, increase in protein during second year may be on the expense of oil accumulation. Thus, finding of the present study are in conformity with above conclusion.

Glucosinolates in both the cultivars tested has been observed to be higher than recommended level for canola types *brassica* cultivars. The most probable reason could be growing of canola types in the same field, where other traditional rapeseed varieties were grown. Thus, cross pollination occurred, which ultimately gave higher

Fig. 1. Relationship between oil and protein during first year

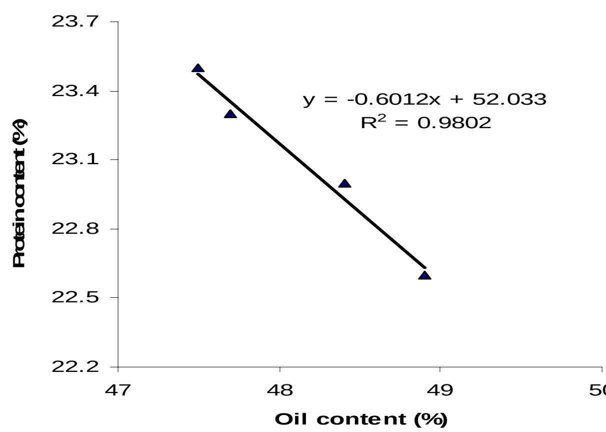


Fig. 2. Relationship between oil and protein during year

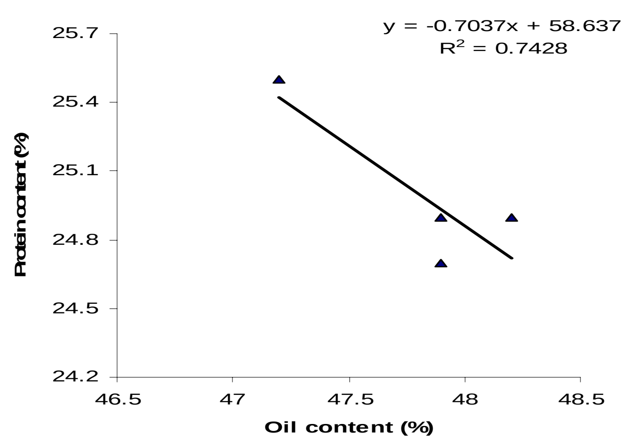


Fig. 3. Relationship between sulphur levels and glucosinlates

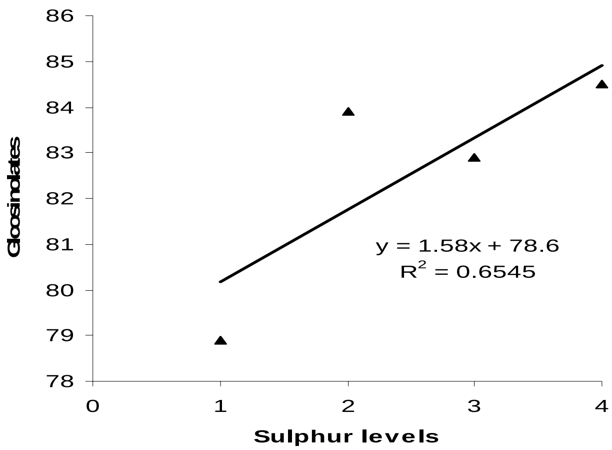
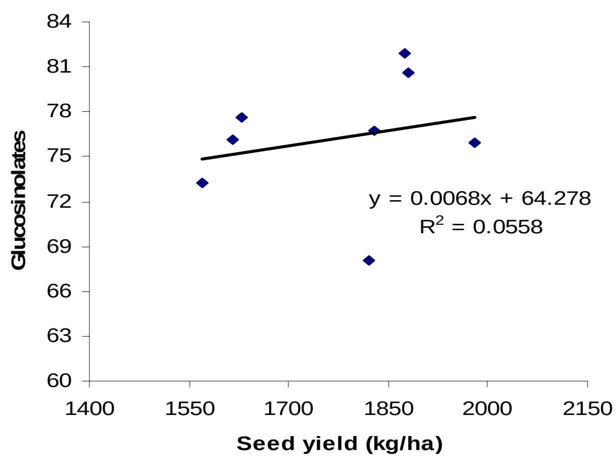


Fig. 4. Relationship between seed yield (kg/ha glucosinolates



glucosinolates. Inconsistent response of sulphur for glucosinolates in our study is in line with Schnug (1997), who reported that the application of 40 and 80 kg ha^{-1} S yielded no significant differences for the glucosinolate content of oilseed rape. However, linear relationship was found between sulphur fertilization and glucosinolate content of mustard. In present study, the linear relationship (Fig. 3) between sulphur levels and glucosinolates during second year is also in line with above conclusion. However, glucosinlates did not depict any significant relationship (Fig. 4) with seed yield. It may be concluded from above results that seed yield would mainly depend upon the availability of moisture during crop life cycle. Similarly, composition of canola seeds would mainly depend upon moisture and prevailing temperature during reproductive stages. However, yield and seed composition may be affected by sulphur application if larger quantities are used on severe deficient soils.

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