

Effects of Sulphur on Fatty Acid Accumulation in *Brassica* Cultivars

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ABSTRACT

Sulphur is the fourth major nutrient in crop production. Most of the crops require as much sulphur as phosphorus. Field experiments were conducted at the University of Arid Agriculture, Rawalpindi, during 2003 - 04 and 2004 - 05 to document the effects of sulphur on fatty acid accumulation in *Brassica* cultivars. Cultivars exhibited statistically significant differences for oleic and linolenic but non-significant differences for erucic acid. Non-significant differences between cultivars for erucic acid may be attributed that cultivars were having similar genetic make up. Sulphur levels effects on fatty acid were narrow and inconsistent except on erucic acid. Inconsistence effects could be attributed to the lower doses of Sulphur used in the study. However, interactive effects were observed to be significant. Oleic acid showed reduction during second year contrary to linolenic and erucic acid those increased during second year as compared to first year. Higher values of erucic acid observed during second year could be attributed to cross pollination of double low cultivars with traditional mustard cultivars grown in the nearby fields. Both the cultivars during two year depicted inverse relationship between oleic and linolenic acid. Similarly, sulphur level affected oleic and erucic acid inversely.

Key Words: *Brassica*; Sulphur; Erucic acid; Oleic acid; Linolenic acid

INTRODUCTION

The non-true oilseed crops like cotton, maize, etc. are contributing up to 73% toward the national edible oil production in the country. The conventional oilseeds (rapeseed & mustard) rank second and contribute about 18 - 20% in the domestic edible oil production. Rapeseed and mustard, the conventional oilseed crops, impose health concerns because of the presence of erucic acid and glucosinolate in meal.

Introduction of canola (*Brassica napus* L) in Pakistan has shown success at many places. It is low in erucic acid and hence is suitable cooking oil. During 2004 - 05 area under canola was 265000 acres with an oilseed production of 159000 tonnes (GOP, 2005). Canola oil has a lower level of saturated fats (only 6%) than any other edible vegetable oil and also has a high proportion of un-saturated fat containing a favorable mix of both mono and polyunsaturated fats. Relative proportion of different fatty acid determines the quality of edible oil. Higher percentage of polyunsaturated fatty acid is considered beneficial for lowering cholesterol in human body (Cunnae, 1995).

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), glucosinolate content and erucic acid (Marschner, 1986). Although variation in erucic acid content is low due to interactions between S and N, the influence of S on fatty acid synthesis should always be considered along with the N supply. In this context, Zhao *et al.* (1997) found a strong interaction between N supply and the proportion of the seed S.

Sulphur is the fourth major nutrient in crop

production. Most of the 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 in cruciferae for the synthesis of volatile oil (Marschner, 1986).

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). Rapeseed (*Brassica campestris* L.) was observed to require 3 - 10 times more sulphur than barley (Bole & Pittman, 1984). 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. Nutritionally, oilseed rape and *Brassica* species in general require S during their growth, for the synthesis of both protein and naturally occurring glucosinolates.

Canola cultivars were introduced in Pakistan during last decade so not much research work has been done particular on quality aspects of the produce (fatty acids composition). 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 effect on fatty acid composition of canola cultivars.

MATERIALS AND METHODS

The experiments to evaluate sulphur effects on quality of oil in *Brassica* (Canola) genotypes were conducted at the

University of Arid Agriculture, Rawalpindi, Pakistan during 2003 - 04 and 2004 - 05. The experimental area lies 33° 38' N and 73° 04' E. The Physical and chemical properties of the site are given in Table I.

The experiments were laid out in a split plot arrangement with cultivars in main plots and sulphur levels in sub-plots replicated four times in net plot size of 5 m x 2.7 m. 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. Seeds of cultivars Shiralee (C₁) and Con- 11 (C₂) were drilled with hand drill using seed rate of 5 kg ha⁻¹. Elemental S [S₁ = 0 (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. 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. Fatty acid composition was 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% level of probability using LSD (Steel & Torrie, 1980). Weather data of the experimental site was recorded, which is given in Table V.

RESULTS

Cultivars exhibited significant differences for oleic acid. The cultivar Shiralee accumulated higher oleic acid as compared to Con- II during both the years (53.8 & 50.6%, respectively). Oleic acid in both the cultivars decreased during 2004 - 05 as compared to that of 2003 - 04. Cultivar x year effects on oleic acid, were observed to be statistically significant. Sulphur levels showed statistically significant differences for oleic acid but effects were not consistent (Table II). Sulphur x year interaction was observed to be significant. The maximum oleic acid (53.7%) was observed from control during first year, while 51.71%, for S₂ during second year. Oleic acid decreased during second year as compared to that observed during first year in all sulphur levels. The maximum (9%) decrease was recorded for control and the least (5%) for S₃. Cultivar x Sulphur interaction exhibited significant but inconsistent effect on oleic acid (Table II). Oleic acid of all interactions decreased in second year as compared to first year. The maximum (11 & 14%) reductions were recorded for C₁ x S₁ and C₂ x S₂, respectively. Cultivar x sulphur x year interaction exhibited significant differences. Overall year effects were recorded to be statistically significant. Higher oleic acid concentration

was recorded during 2003 - 04 as compared to that of 2004 - 05.

Cultivars exhibited non-significant differences for linolenic acid (Table III). Cultivar x year interaction showed statistically significant differences. The cultivar Con-II accumulated higher linolenic acid during 2003 - 04. However, during 2004 - 05 Shiralee took over the position with narrow differences. Linolenic acid in both cultivars increased during second year as compared to that observed during first year. Sulphur level showed statistically significant effects on linolenic acid. During, 2003 - 04 and 2004 - 05, control gave the highest values (8.93 & 9.24%, respectively) but differences among rest of the treatments were not symmetrical. Cultivar x sulphur interaction exhibited significant differences. The control with both the cultivars (C₁ x S₁ & C₂ x S₁) remained at the top for linolenic acid during first year. However, during second year C₁ x S₄ and C₂ x S₃ took over the top position with 10.95 and 11.25% linolenic acid, respectively. Sulphur x cultivar x year interaction exhibited significant differences. Linolenic acid increased during 2004 - 05 as compared to that observed during 2003 - 04 in all the interactions. Linolenic acid accumulated during 2004 - 05 was significantly higher than that observed during 2003 - 04.

Cultivars exhibited non-significant differences for erucic acid. The Cultivar Con-II accumulated higher erucic acid as compared to Shiralee (Table IV). Erucic acid accumulation increased in both cultivars during 2004 - 05 as compared to that recorded during 2003 - 04. However, accumulation of erucic acid was observed to be higher in Con- II as compared to Shiralee. Interaction of cultivar x year was observed to be statistically non-significant. Sulphur levels affected erucic acid significantly; however, effects were not consistent. The maximum (28%) increase of erucic acid was recorded for control. Sulphur x year effects were observed to be statistically non-significant. The maximum (46.65 & 51.66%) erucic acid, was recorded for S₂ and S₃ during two years of experimentation, respectively (Table IV). However, minimum erucic acid (38.89%) was observed for control during first year, while S₄ gave the minimum erucic acid (48.71%) during second year. Cultivar x sulphur interaction was revealed to be non-significant. The C₂ x S₃ interactions gave the highest erucic acid (45.8 & 53.3%, respectively) during both the years. Years affected erucic acid significantly with higher value observed during 2004 - 05 as compared to those recorded during 2003 - 04.

DISCUSSION

Sulphur mainly enhances the reproductive growth and the proportion of the reproductive tissues (inflorescences & pods) in total dry matter (McGrath & Zhao, 1996). Under S deficient conditions, the amount of amino acids and nitrates in leaves increases dramatically (Hue *et al.*, 1991) and

Table I. Physical and chemical properties of experimental site

Clay	Silt	Sand	B. D	pH	EC	O.M	N	P	K	SO ₄ -S
15%	45%	40%	1.45 Mg cm ⁻³	7.7	0.25 d S m ⁻¹	0.66%	3.84 ug g ⁻¹	5.2 mg kg ⁻¹	120 mg kg ⁻¹	7.6 mg kg ⁻¹

Table II. Effect of sulphur on oleic acid (%) of two *Brassica* cultivars

Cultivars	Shiralee (C1)			Con-II (C2)			Cultivars Mean
Sulphur Levels	2003-04	2004-05	Mean	2003-04	2004-05	Mean	
S1 (0)	56.88	50.58	53.73	51.73	48.63	50.18	(C1) 52.17
S2 (15)	51.08	50.80	50.94	55.53	47.90	51.71	(C2) 50.44
S3 (20)	53.90	50.68	52.29	49.80	47.68	48.74	
S4 (25)	53.53	50.93	52.23	54.23	48.08	51.15	
Mean	53.79	50.56		52.82	48.07		

LSD (0.5%)

Cultivars : 1.61, Sulphur = 1.560, Interactions (Cultivar x year = 1.357,
Sulphur x year = 1.103, Cultivar x sulphur = 2.207, Cultivar x sulphur x year = 1.560)**Table III. Effect of sulphur on linolenic acid (%) of two *Brassica* cultivars**

Cultivars	Shiralee (C1)			Con-II (C2)			Cultivars Mean
Sulphur Levels	2003-04	2004-05	Mean	2003-04	2004-05	Mean	
S1 (0)	7.08	10.78	8.93	7.58	10.90	9.24	(C1) 8.50
S2 (15)	5.58	10.90	8.24	7.18	10.83	9.00	(C2) 8.97
S3 (20)	5.60	10.33	7.96	6.78	11.25	9.01	
S4 (25)	6.78	10.95	8.86	7.45	9.73	8.59	
Mean	6.26	10.74		7.24	10.70		

LSD (0.5%)

Cultivars : 0.77, Sulphur = 0.778, Interactions (Cultivar x year = 0.675,
Sulphur x year = 0.550, Cultivar x sulphur = 1.101, Cultivar x sulphur x year = 0.778)**Table IV. Effect of sulphur on erucic acid (%) of two *Brassica* cultivars**

Cultivars	Shiralee (C1)			Con-II (C2)			Cultivars Mean
Sulphur Levels	2003-04	2004-05	Mean	2003-04	2004-05	Mean	
S1 (0)	36.73	48.15	42.44	41.05	51.25	46.15	(C1) 44.35
S2 (15)	44.78	47.53	46.15	37.73	52.10	44.91	(C2) 46.34
S3 (20)	40.28	50.05	45.16	45.78	53.28	49.53	
S4 (25)	39.53	47.80	43.66	40.78	49.63	45.20	
Mean	40.33	48.38		41.33	51.56		

LSD (0.5%)

Cultivars : 2.87, Sulphur = 4.466, Interactions (Cultivar x year = 3.101,
Sulphur x year = 3.11, Cultivar x sulphur = 6.315, Cultivar x sulphur x year = 4.446)**Table V. Rainfall and temperature at experimental site**

2003-04	Mean Maximum	Mean minimum	Total Rainfall
Month	Temperature °C	Temperature °C	(mm)
Oct, 2003	30.2	12.3	2.90
Nov, 2003	23.9	3.4	17.3
Dec, 2003	17.8	3.5	45.0
Jan, 2004	14.5	5.1	91.2
Feb, 2004	25.30	6.0	37.0
Mar, 2004	31.6	12.5	Traces
2004-05			
Oct, 2004	27.8	12.6	80.8
Nov, 2004	25.6	6.8	19.8
Dec, 2004	20.1	4.1	25.2
Jan, 2005	17.7	4.6	86.2
Feb, 2005	22.3	6.2	37.0
Mar, 2005	30.4	11.9	0.00
April, 2005	34.7	17.5	8.2

protein degradation within chloroplasts occurred (Dannehl *et al.*, 1995). Besides, sulphur affects photosynthetic characteristics. Thus limits protein synthesis by limiting the amount of methionine and cysteine available for the assembly of new proteins (Sexton *et al.*, 1997). Oilseed rape that grows on S-limiting soils will suppress the development of reproductive organs and even lead to pod abortion (Zhao *et al.*, 1997). 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 seeds weight, while protein and oil may compete for the remaining space in seeds. Similarly, proportion of different fatty acids may change in the oil.

In present study, differences between cultivars for oleic acid may be due to genetic make up. In-consistent response of oleic acid to sulphur levels is in line with findings of Ahmed and Abdin (2000), who reported non-significant differences among sulphur levels for oleic acid, however, oleic acid recorded in our study are higher than those reported by Ahmed and Abdin (2000), which may be cultivars differences or field conditions. Climatic variability during both the years was wide and noticeable. A total of 193 mm of rainfall was received during first year, while 247 mm during second year (Table V). Thus, interaction of field conditions, S and years would have affected oleic acid accumulation. Linolenic acid in present study was affected by sulphur levels but differences were narrow, which depicts that it is least affected by S. However, climatic conditions would have affected and caused an increase during second year. Ample moisture availability and low average temperature during seed development might have given higher linolenic acid during second year. Both the cultivars during two years depicted an inverse relationship (Fig. 1) between oleic and linolenic acid. An inverse relationship between monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic acid) in sunflower has been reported by Flagella *et al.* (2002), which was mainly attributed to maturing of crop at low temperature. Thus, findings of present study are consistent to those of Flagella *et al.* (2002). The inverse relationship between two fatty acid also provide the clue that within oil space covered by chain of different fatty acids changes inversely being influenced by environmental factors, mainly temperature and moisture availability.

Major changes occur in oleic acid and erucic acid content during seed development. The pattern of change of erucic acid may be consistent with the chain elongation of oleic acid to give erucic acid. Supply of S in portions during seed development reduced the conversion of oleic acid (18:1) to erucic acid (22:1). Leading to the reduced 22:1/18:1 ratio and thus, improved the quality of the oil (Ahmed & Abdin, 2000). Significant inverse relationship (Fig. 2a & b) between oleic and erucic acid in response to S during both the cropping seasons are in line with above conclusion. However, Jan *et al.* (2002) found significant

Fig. 1. Relationship between oleic and linolenic acid of both cultivars

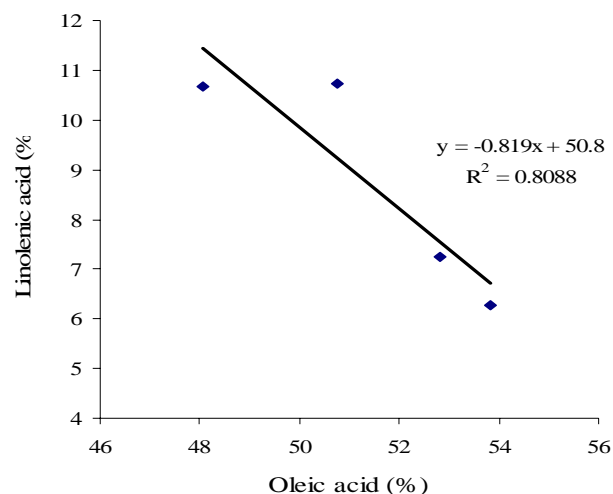
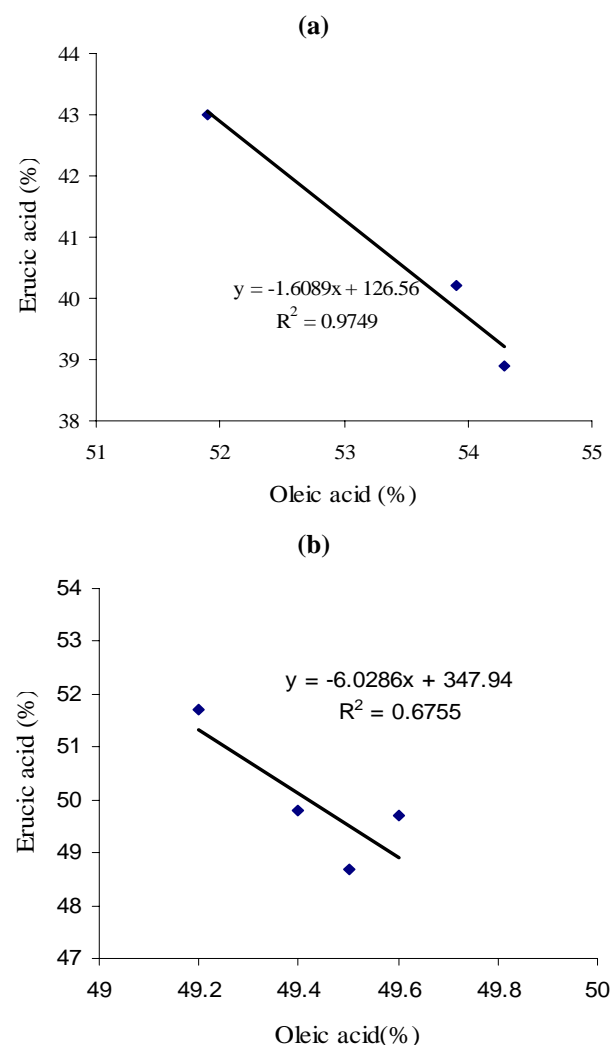


Fig. 2. Relationship between Oleic and Erucic acid during (a) 2003-04 and (b) 2004-05



effect of S on erucic acid with highest value from treatment, which received 90 kg S ha⁻¹. Though in present study, S levels affected erucic acid significantly, yet erucic acid values were not consistent to S rates. This might be due to lower rates used in our study. Severe sulphur deficient soils usually do not respond to smaller amounts as major portion of applied S is held in the soil and less available to plants (Zhao *et al.*, 1997). The experiments of present study were conducted at a site, which is severely deficient of S as per critical limits given by Ahmad *et al.* (1994), thus response was not consistent and non-significant. So, findings are in line with above conclusion. Overall higher values of erucic acid may be attributed to the cross pollination of double low cultivars with traditional mustard cultivars grow in nearby fields.

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