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Soybean seed protein, oil, and fatty acids are altered by S and S + N fertilizers under irrigated or non-irrigated environments*

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ABSTRACT

Information on the effect of sulfur (S) or sulfur+nitrogen (S + N) on soybean seed composition is scarce. Thus, the objective of this study was to investigate the effects of S, and S + N fertilizers on soybean [(*Glycine max* (L.) Merr.)] seed composition in the Early Soybean Production System (ESPS) under irrigated (I) and non-irrigated (NI) environments. Two separate field experiments were conducted from 2005 to 2007. One experiment was irrigated, and the second experiment was non-irrigated. Under I condition, S at a rate of 44.8 kg/ha alone or with N at 112 kg/ha resulted in a consistent increase in seed protein and oleic acid concentrations, and a decrease in oil and linolenic acid concentrations compared with the control (C). For example, in 2006 and compared with the C, application of S + N increased the percentage up to 11.4% and 48.5% for protein and oleic acid, respectively. However, oil concentration decreased by 3%. Protein and oleic acid increase were accompanied by a higher percentage of leaf and seed N and S. Under NI conditions, seed protein and oleic acid concentrations were significantly higher in C than in any S or S + N treatments, but the oil and linolenic acid concentrations were significantly lower. The results indicate that specific rate of S alone or S + N combined can alter seed composition under irrigated or nonirrigated conditions. This knowledge may help plant breeders to develop and release cultivars to suit specific target locations to grow new value-added soybeans or select for specific seed composition traits under specific environmental stress factors such as drought.

Keywords: Fatty Acids; Nutrition; Oil; Protein; Seed Composition; Soybean

*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

1. INTRODUCTION

Soybean is a major source of high nutritional quality protein and oil [1], and its economic value can be determined by seed protein and oil content. Protein in soybean seed ranges from 341 to 568 g/kg with a mean of 421 g/kg, and oil ranges from 83 to 279 g/kg with a mean of 195 g/kg [2]. The concentration of saturated fatty acids in soybean oil ranges from 100 to 120 g/kg for palmitic acid and from 22 to 72 g/kg for stearic acid [3]. The mean concentration of unsaturated fatty acids in oil is 240 g/kg for oleic acid, 540 g/kg for linoleic acid, and 80 g/kg for linolenic acid [4].

In the midsouthern USA, soybean is produced under both irrigated and non-irrigated conditions, with about 50% of soybean grown in the Mississippi Delta non-irrigated. This is mainly because soybean yield varies from season to season due to varying environmental conditions such as soil type, rainfall, temperature, and management practices [5]. To avoid drought stress during late July through early September, the Early Soybean Production System (ESPS) in the midsouth was developed using early maturing group cultivars (MG IV and V) planted in April through early May and harvested in August and September [6,7]. Although the yield in ESPS has been shown to be higher under both irrigated and non-irrigated environments compared to the traditional production system, its effects on seed composition have not been thoroughly evaluated [8]. The change from conventional soybean production system to ESPS has shifted the time of oil and protein deposition to a warmer period, leading to possible change in the accumulation of protein, oil, and fatty acid rates. In the Mississippi Delta, USA, sulfur fertilizer is not commonly applied to the soybean crop because Delta soil has adequate nutrients. In general, 10 units of nitrogen used by the plants require one unit of sulfur, therefore, continuous removal of sulfur from soil or inhibition of sulfur uptake due to drought or high temperature may alter soybean seed composition (protein, oil, and fatty acids) since both N and S are essential components in seed composition constituents.

The physiological and biochemical roles of N and S have been previously reported for plants [9,10]. Seed N concentration is correlated with N availability in plants, and the contribution of N remobilization to seed N accounted for 80% to 90% of total N in soybean [11]. Depending on growth stages [12], seed N content differed significantly between R5, R6, and R7 growth stage, and the mean N contents were 6.28% at R5, 6.35% at R6, and 6.68% at R7 [13]. The majority of N demand takes place at the R5 developmental stage [14-16], when seeds become the nutrient sink, and remobilization and translocation of N from vegetative tissues into the developing seeds occurs.

Relationships between seed composition and nutrient levels in soil and seed were previously reported [17]. For example, higher percentages of protein and oleic acid were accompanied by higher concentrations of N, K, B, and Zn in seed [17]. Recently, it was found that foliar boron application increased seed protein and oleic acid concentrations [18,19]. The effect of large amounts of fertilizer N on soybean protein and oil concentrations was previously studied [7]. In their study, a significant decrease in seed protein concentration (2.7% and 1.9%), an increase in oil concentration (2.2% and 2.7%), and a decrease in the protein/oil ratio (4.7% and 4.6%) under irrigated and non-irrigated environments, respectively [7] were found.

In spite of the well-established role of N and S nutrition in plant growth and development, there is a lack of information on the effect of S alone or S + N combined on soybean seed composition in ESPS under irrigated and non-irrigated environments. Therefore, the objective of this study was to investigate the effects of S alone or S combined with N fertilizer on soybean seed protein, oil, fatty acids, and the physiological dynamics of leaf and seed S and N concentration under irrigated and non-irrigated conditions in ESPS.

2. MATERIALS AND METHODS

Two field experiments were conducted at the Delta Research and Extension Center, Stoneville, MS (33˚26'N latitude) in 2005, 2006, and 2007. One experiment was irrigated (I) and the second experiment was non-irrigated (NI)), and both experiments were conducted as previously described [6,20]. Experimental conditions for both studies were similar, except irrigation. Treatments were separated by border of 5 rows of soybean with an alley of 7.3 meters. Soybean cultivar AG3905, late maturity group III, was planted on 17 May 2005, 17 May 2006, and 23 April 2007. Row spacing was 0.66 m and seeding rate was 27 seeds m^{-1}. Plots were 5 rows wide and 4.88 m long. Soybean was harvested on 14 October 2005, 3 October 2006, and 21 September 2007. When soybeans reached R8 growth stage, the middle three rows were harvested from each plot for seed [12]. The soil was a silty clay loam with a texture of 16% sand, 44% silt, and 40% clay, with 2.17% organic matter, 0.9 g N/kg, and 92.30 mg S/kg. Different rates of S and S combined with N fertilizer were supplied as follows:

Control (C) = 0 kg·S/ha + 0 kg·N/ha; Treatment 1 (T1) = 22.4 kg·S/ha + 0 N; Treatment 2 (T2) = 44.8 kg·S/ha + 0 N; Treatment 3 (T3) = 67.2 kg·S/ha + 0 N; Treatment 4 (T4) = 0 kg·S + 112 kg·N/ha; Treatment 5 (T5) = 22.4 kg·S/ha + 112 kg·N/ha; Treatment 6 (T6) = 44.8 kg·S/ha + 112 kg·N/ha; Treatment 7 (T7) = 67.2 kg·S/ha + 112 kg·N/ha. Borders from each side of each plot to avoid contamination and carry-over to other plots were used. Sulfur and S + N were broadcasted at V3-V4 growth stage.

The experiment was furrow irrigated as needed to avoid severe water stress soil-water potential for irrigated soil ranged between 0 and −21 kPa over the growing season. For non-irrigated plots, the soil-water potential, especially during the critical stages of the crop development in June to August was as low as −191 kPa, depending on the year.

2.1. Experimental Design and Analysis

Each experiment was a randomized complete block design, with S and N fertilizer levels. Four replicates in each irrigation treatment were used. Analysis of variance using Proc GLM was conducted using SAS [21], with level of significance of $P \leq 5\%$.

2.2. Soil and Seed Mineral Analyses

Analyses of soil and seed were conducted at The University of Georgia's Soil, Plant, and Water Laboratory, Athens, GA. Soil mechanical analysis and organic matter, using a loss on ignition method for 3 hours at 360˚C, were conducted. Fully expanded leaves were collected at R5-R6 stage, and seed samples were collected at full maturity. Nitrogen and S were analyzed in 0.25 g of leaf and seed samples using an elemental analyzer (LECO CNS-2000, LECO Corporation, MI, USA).

2.3. Protein, Oil, and Fatty Acid Analysis

Seeds were sampled from each treatment and analyzed for seed composition using a near-infrared (NIR) reflectance diode array feed analyzer (Perten, Springfield, IL) for protein, oil, and fatty acids [22,23]. The calibration equation was developed by the University of Minnesota, using Perten's Thermo Galactic Grams PLS IQ software (Springfield, IL). The analysis was performed on the basis of dry matter [22,24]. Throughout the study, we used (g constituent/kg seed dry weight) to refer to "concentration" of a constituent. We used "kg constituent/ha" to refer to the total constituent based on seed yield per ha.

3. RESULTS

3.1. Analysis of Variance: Seed Yield and Seed N and S

Under I conditions, there was no significant effect of S, N, or S + N supply (Fert) on yield, as expected (**Table 1**). Year was the main source of yield variability (**Table 1**). However, Fert was significant for the percentage of N and S in seed and leaf as well as, total N and S (kg/ha) (**Table 1**). Year was significant for seed N/S ratio and seed total S (**Table 1**). Year × Fert interaction was significant for total N only. Under NI, there was no effect of Fert on yield. Year was significant for seed S percentage, N/S ratio, and total S and N. Fert was significant for seed N/S ratio only. There was no year × Fert interaction for seed or leaf N or S (**Table 1**).

3.2. Analysis of Variance: Seed Protein, Oil, and Fatty Acids

Under I conditions, Fert effect was significant for protein, oil, oleic and linolenic acid concentrations (g constituent/kg seed) (**Table 2**). Year was significant for seed protein, oil, palmitic, stearic, oleic, and linolenic acid concentrations (**Table 2**). Year × Fert interactions were significant for protein, oleic, and linolenic acids concentrations. Under NI, year was significant for all seed composition constituents (**Table 2**). Fert was significant for protein, oil, palmitic, and linolenic acid concentrations (**Table 2**). Year × Fert interactions were significant for protein, oleic, and linolenic acids concentrations (**Table 2**). Total (kg/ha) seed composition cons

Table 1. Analysis of variance (source of variability, F value, and level of significance) for the effect of year, fertilizer [Fert (S and N supply)], and their interactions on seed N and S percent and seed N/S ratio and total seed N and S (kg·N or S/ha), and on leaf N and S percentage and leaf N/S ratio under irrigated and non-irrigated conditions at Stoneville, MS 2005-2007[a].

					Irrigated				
	Yield	Seed					Leaves		
		N %	S %	ratio	N (kg/ha)	S (kg/ha)	N %	S %	ratio
Source	F value	F value	F value		F value	F value	F value	F value	
Year	109***†	ns	ns	8.03**	ns	8.1***	ns	ns	ns
Fert	ns	4.7***†	16.8***	3.8*	4.8*	10.6*	6.7**	4.29*	ns
Year × Fert	ns	ns	ns	ns	4.8*	ns	ns	ns	ns
Rep (year)	14.9***	ns	ns	ns	ns	ns	ns	ns	ns
					Nonirrigated				
	Yield	N %	S %	ratio	N (kg/ha)	S (kg/ha)	N %	S %	ratio
Source	F value	F value	F value		F value	F value	F value	F value	
Year	68.7***†	ns	4.49*	8.94**	66.2***	55.76***	ns	ns	ns
Fert	ns	ns	ns	3.03*	ns	ns	ns	ns	ns
Year × Fert	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep (year)	13.1***	ns	ns	ns	11.9***	6.4***	ns	ns	ns

[a] *Significance at $P \le 0.05$; **Significance at $P \le 0.01$; ***Significance at $P \le 0.001$.

Table 2. Analysis of variance (source of variability, F value and level of significance) for the effect of year, fertilizer [Fert (S and N supply)], and their interactions on seed protein, oil, and fatty acid concentrations (g of constituent/kg dwt) under irrigated and non-irrigated conditions at Stoneville, MS 2005-2007[a].

Source	Protein	Oil	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)
Irrigated							
Year	96.4***	11.7***	28.9***	300***	12.9***	6.9***	53.5***
Fert	23.6***	3.5***	ns	ns	36.1***	ns	4.4***
Year × Fert	3.1***	ns	ns	ns	4.0***	ns	3.4***
Rep(year)	3.7***	ns	ns	5.5***	ns	2.9**	ns
Non-irrigated							
Year	46.1***	29.6***	212***	532***	19.1***	26.5***	6.87**
Fert	55.1***	8.5***	2.2*	ns	39.6***	ns	12.1***
Year × Fert	2.0*	ns	ns	ns	2.1*	ns	3.2***
Rep (year)	ns	2.5*	4.2***	ns***	ns	2.7**	ns

a*Significance at $P \leq 0.05$; **Significance at $P \leq 0.01$; ***Significance at $P \leq 0.001$.

Table 3. Analysis of variance (source of variability, F value, and level of significance) of the effects of year, fertilizer [Fert (S and N supply)], and their interactions for total seed protein, oil, and fatty acids (kg of constituent/ha) under irrigated and non-irrigated conditions for 2005, 2006, and 2007[a].

Source	Protein	Oil	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)
Irrigated							
Year	114*** †	129.6***	21.1***	167***	105***	87.4***	88.7***
Fert	3.0**	ns	ns	ns	11.9***	ns	3.7**
Year × Fert	ns	ns	ns	ns	ns	ns	ns
Rep (year)	14.0***	14.6***	10.2***	9.5***	12.7***	15.7***	5.2***
Non-irrigated							
Year	48.7***	68.1***	27.8***	326***	49.9***	47.5***	44.5***
Fert	2.2*	ns	Ns	ns	6.3***	ns	5.4***
Year × Fert	ns	2.0*	1.9*	ns	ns	ns	2.9**
Rep (year)	11.0***	9.2***	6.1***	16.9***	10.8***	12.3***	4.9***

a*Significance at $P \leq 0.05$; **Significance at $P \leq 0.01$; ***Significance at $P \leq 0.001$.

tituents showed that under I conditions, year and Fert effects were significant for total seed constituents and showed similar trend as those of concentrations, except for total oil, where Fert had no significant effect (**Table 3**). No year × Fert effect was shown for total seed constituents (**Table 1**). Total seed constituents under NI showed that year and Fert had similar trend as those observed in seed concentrations, except that Fert had no significant effect for oil and palmitic acid. Fert × year interactions were significant for oil, palmitic, and linolenic acid (**Table 3**), differing from those of concentration.

3.3. Mean Values: Yield and Seed Composition as Affected by S and S + N Supply (Fert)

No clear trend or significant effects of S, N, or S + N on yield were observed under I or NI conditions (**Figure 1**). Under I conditions, seed protein and oleic acid concentrations in 2005 were higher in T2 and T6 compared to C. Linolenic acid had the opposite trend of oleic acid under those treatments (**Table 4**). Seed oil was greater in C than in T6, and lowest in T1, T2, and T3 treatments. No clear trend of the effect of S or S + N supply on stearic, palmitic, and linoleic acids was observed. Total protein, oleic, and linolenic acid showed the same trend as those of concentrations under I conditions (**Table 4**).

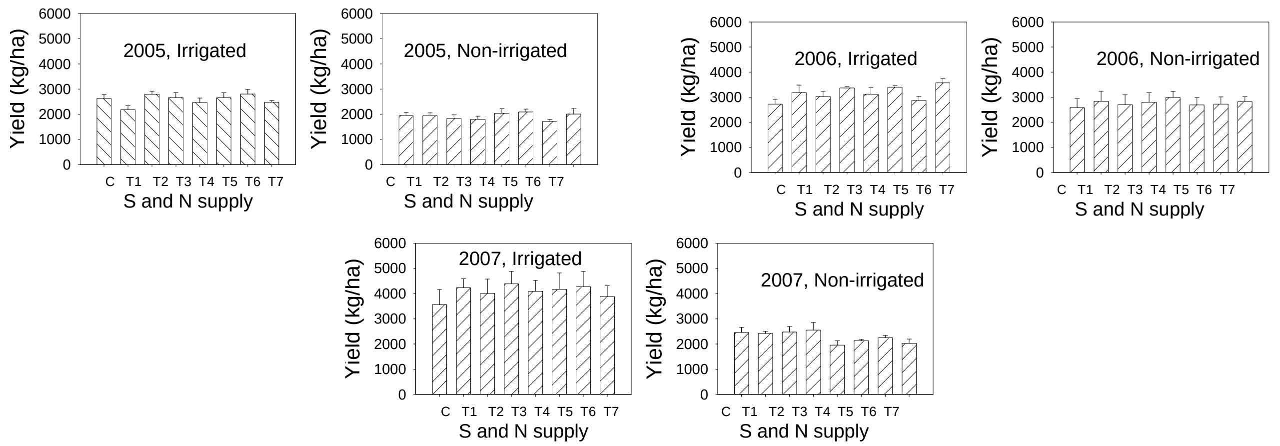

Figure 1. Effect of S or S and N combined on soybean seed yield under irrigated and non-irrigated conditions in 2005 through 2007. Treatments were: Control (C) = 0 kg·S/ha + 0 kg N/ha; Treatment 1 (T1) = 22.4 kg·S/ha + 0 N; Treatment 2 (T2) = 44.8 kg·S/ha + 0 N; Treatment 3 (T3) = 67.2 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S + 112 kg·N/ha; Treatment 5 (T5) = 22.4 kg·S/ha + 112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha; Treatment 7 (T7) = 67.2 kg·S/ha + 112 kg·N/ha.

Table 4. Mean values of protein, oil, and fatty acid concentrations (g constituent/kg) and total (kg constituent/ha) as affected by S and N supply (T1-T7) treatments (Treat) under Irrigated (I) and Non-irrigated (NI) Conditions in 2005[a].

Concentration (g/kg)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	417	203	136	33.3	214	559	62.3	460	176	140	38.5	296	527	54
T1	441	186	130	34.3	227	554	60.8	430	190	143	37.5	254	535	60
T2	457	189	133	33.5	299	560	47.8	438	197	138	37.8	253	527	56
T3	452	186	133	35.5	224	548	62.8	428	198	136	37.3	257	532	58
T4	442	191	140	35.0	207	558	60.3	416	199	142	36.5	243	545	75
T5	442	193	134	33.8	204	566	65.0	418	199	135	37.5	233	524	73
T6	453	194	136	34.0	284	548	56.8	417	198	136	37.0	242	526	77
T7	451	194	134	34.8	213	559	62.5	410	202	141	36.3	247	533	72
LSD	4.92	3.74	2.27	1.74	8.40	9.56	2.55	4.36	3.26	2.6	1.3	4.50	10.5	3.92

Total (kg/ha)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	1097	535	355	86.8	561	1471	165	897	342	273	74.8	577	1029	105
T1	962	403	281	73.9	495	1208	133	832	367	277	72.7	491	1033	118
T2	1277	528	372	93.4	839	1566	134	799	358	253	68.9	461	967	102
T3	1205	494	354	94.4	595	1462	167	770	355	245	67.3	460	955	104
T4	1090	469	343	85.4	506	1377	149	848	405	289	74.6	497	1107	153
T5	1175	513	355	88.5	542	1506	173	870	415	281	78.6	487	1088	152
T6	1270	544	380	95.0	795	1534	159	714	340	232	63.5	414	902	131
T7	1117	480	330	85.6	525	1386	154	822	406	281	71.7	493	1073	146
LSD	75.7	30.1	18.4	4.3	43.0	98.9	12.5	59.81	27.63	18.6	5.6	36	77.6	13.06

[a]control (C) = 0 kg·S/ha + 0 kg N/ha; Treatment 1 (T1) = 22.4 kg·S/ha + 0 N; Treatment 2 (T2)= 44.8 kg·S/ha + 0 N; Treatment 3 (T3) = 67.2 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S + 112 kg·N/ha; Treatment 5 (T5) = 22.4 kg·S/ha + 112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha; Treatment 7 (T7) = 67.2 kg·S/ha + 112 kg·N/ha.

In 2006 and under I, protein and oleic acid concentrations were higher when 44.8 kg·S/ha was used (T2 and T6) compared to C or other treatments (**Table 5**), following the same pattern as those in 2005. Linolenic acid was the lowest in T2, T6, and T7. It is clear that both protein and oleic acid followed the same trend of that of 2005, but the trend of oil, linoleic and linolenic acids was inconsistent with those of 2005 (**Table 5**). Mean values for total seed protein and oleic acid under I conditions were the lowest in C compared with any of S or S + N treatments (**Table 5**).

In 2007 and under I, protein concentration was higher in all Fert treatments compared to C, and T2 and T6 had the highest protein concentrations (**Table 6**). Oleic acid was highest in T2, T6, and T7. Total seed protein and total oleic acid showed similar trends as those of protein and oleic acid concentrations (**Table 6**).

Under NI, protein concentration was higher in C compared to all S or S + N treatments. For example, in 2005, depending on the S or S + N treatment, the percentage increase from the control ranged from 4.8 to 10.9% for protein and from 14.2% to 21.3% for oleic acid. The decrease in oil ranged from 8% to 14.8% and from 0% - 42.6% for linolenic acid. This trend was also consistent for 2006 and 2007 (**Tables 5** and **6**). Total protein and oleic acid showed the same trend as those of concentrations (**Tables 4-6**).

Table 5. Mean values of protein, oil, and fatty acid yield (g constituent/kg) and total (kg/ha) as affected by S and N Supply (T1-T7) Treatments (Treat) † under Irrigated (I) and Non-irrigated (NI) Conditions in 2006[a].

Concentration (g/kg)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	402	198	132	48.8	214	48.8	102	453	174	107	55.0	292	508	55.5
T1	411	200	133	50.0	211	50.0	103	404	199	118	55.0	257	509	59.5
T2	447	191	129	49.0	290	49.0	98.9	421	211	116	56.3	258	508	62.0
T3	411	189	140	50.5	200	47.0	105	400	198	114	55.3	240	497	89.5
T4	401	193	130	50.0	218	50.0	95.8	394	202	124	54.2	235	509	90.0
T5	412	193	115	50.0	228	50.0	91.0	400	201	106	54.5	222	517	82.5
T6	448	192	125	49.5	318	49.5	83.5	394	207	116	54.8	219	521	72.8
T7	421	193	125	47.0	222	47.0	68.8	397	209	108	54.0	223	518	70.0
LSD	5.7	3.6	4.8	1.7	10.3	1.7	4.4	4.41	4.59	5.9	1.3	6.36	7.1	4.68
Total (kg/ha)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	1093	540	358	133	583	1438	275	1167	451	274	142	751	1315	145
T1	1317	637	417	158	676	1707	323	1147	565	334	156	723	1449	173
T2	1354	579	388	149	870	1585	297	1140	567	311	150	693	1380	166
T3	1384	637	472	170	675	1781	353	1112	556	315	154	675	1395	254
T4	1255	601	404	156	686	1653	301	1177	601	367	162	708	1528	265
T5	1398	655	390	170	775	1831	309	1072	536	279	146	598	1400	221
T6	1289	553	359	141	914	1506	240	1070	564	314	148	596	1421	198
T7	1504	690	447	168	797	1949	245	1118	587	305	152	630	1459	196
LSD	88.8	40.9	21.2	10.4	58.9	104	19.4	126.2	60.5	32.6	15.7	77.3	171	24.8

[a]control (C) = 0 kg·S/ha + 0 kg·N/ha; Treatment 1 (T1) = 22.4 kg·S/ha + 0 N; Treatment 2 (T2)= 44.8 kg·S/ha + 0 N; Treatment 3 (T3) = 67.2 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S + 112 kg·N/ha; Treatment 5 (T5) = 22.4 kg·S/ha + 112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha; Treatment 7 (T7) = 67.2 kg·S/ha + 112 kg·N/ha.

Table 6. Mean values of protein, oil, and fatty acids concentration (g constituent/kg) and total (kg constituent/ha) as affected by S and N supply (T1-T7) treatments (Treat) under Irrigated (I) and Non-irrigated (NI) conditions in 2007[a].

Concentration (g/kg)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	428	217	109	43.0	227	537	60.0	451	205	104	44.0	293	536	47.5
T1	455	200	105	41.8	217	549	54.8	428	205	104	43.3	231	535	78.3
T2	459	204	110	43.0	307	529	62.8	415	219	103	43.5	221	545	78.3
T3	444	200	110	43,5	238	568	70.8	409	210	106	44.3	227	533	71.8
T4	442	199	131	45.3	233	568	62.3	405	224	108	44.5	229	536	77.8
T5	453	198	105	42.3	247	545	56.0	403	214	105	43.8	224	529	82.5
T6	464	198	119	44.8	287	564	48.0	406	214	108	43.8	224	535	74.0
T7	448	195	103	41.3	305	547	62.5	391	211	102	43.8	228	537	77.8
LSD	4.9	4.5	9.5	0.94	10.3	23.5	5.1	3.65	6.55	2.2	0.5	6.51	4.4	4.32
Total (kg/ha)														
	Irrigated							Non-irrigated						
Treat	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Protein	Oil	C16:0	C18:0	C18:1	C18:2	C18:3
C	1522	779	394	153	794	1919	216	1108	504	255	108	719	1316	115
T1	1924	844	446	177	922	2330	229	1035	496	252	105	560	1295	190
T2	1844	808	443	171	1057	2137	252	1027	536	254	108	547	1351	192
T3	1947	871	490	192	1057	2497	318	1041	531	273	113	584	1362	185
T4	1804	810	560	186	965	2360	254	792	433	211	87.0	448	1051	154
T5	1884	815	436	173	1019	2292	227	857	454	223	93.0	477	1123	176
T6	1978	838	511	191	1222	2436	204	910	480	241	98.2	504	1202	167
T7	1738	765	403	159	1192	2132	237	790	427	206	88.5	459	1092	158
LSD	228	99.0	81.6	21.3	149	332	37.0	72.10	32.34	20.2	7.7	45.48	100.4	16.89

[a]control (C) = 0 kg·S/ha +0 kg·N/ha; Treatment 1 (T1) = 22.4 kg·S/ha + 0 N; Treatment 2 (T2) = 44.8 kg·S/ha + 0 N; Treatment 3 (T3) = 67.2 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S + 112 kg·N/ha; Treatment 5 (T5) = 22.4 kg·S/ha + 112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha; Treatment 7 (T7) = 67.2 kg·S/ha + 112 kg·N/ha.

3.4. Mean Values: Seed and Leaf S and N as Affected by S and S + N Supply

Under I, percentage (%) and total N and S (kg·N or S/ha) in leaf tissue and seed were higher in both T2 and T6 samples compared to C in 2005 and 2006 (**Tables 7** and **8**). N/S ratio was generally higher in C than T2 or T6. The opposite trend of N/S ratio in leaf was observed, *i.e.*, N/S ratio was generally higher in T2 and T6 compared to the C (**Table 7**). Under NI, seed S percentage was higher in T2 and T6 than in C in 2005 and 2006, but seed N percentage was inconsistent across years (**Table 7**). Under NI, leaf N percentage in C was higher than other S or S + N treatments, and leaf S percentage was inconsistent across years (**Table 7**).

4. DISCUSSION

Seed Yield and Seed N and S

Year was the main source for yield variability, indicating that yield is affected by yearly seasonal environmental factors such as drought and heat. There was no significant yield difference due to S, N, or S + N application in each year, but significant seed composition quality differences were observed under irrigated or non-irrigated. Year, Fert, and year × Fert interactions were the main source for protein, oleic, and linolenic concentrations variability under both I and NI conditions, indicating that the effect of S or S + N supply on seed composition depended on the environmental factors and S or

S + N management in each year. These environmental factors influencing seed constituents could be irrigation/drought or temperature [25-27].

No consistency effects of fertilizer treatments were noticed on seed composition constituents, except for T2 and T6. This could be that a specific rate of S or S + N supply is required to result in seed composition changes. The increase of seed protein and oleic acid under I conditions in T2 and T6 compared to the C in 2005, 2006, and 2007 indicates that applying the rates (44.8 kg·S/ha alone or with N at 112 kg·N/ha) increased the concentrations and total seed protein and oleic acid. This may be due to the indirect effect of S on enzymes involved in *de novo* protein and oleic synthesis or enzyme activities, especially fatty acid desaturases. Also, S may have an indirect effect on S and N uptake and translocation from leaf tissues to seed at vegetative stages. It was reported that seed protein requires a high mobilization of stored vegetative N [28,29] and S [30] to seed. This observation may be supported by the current results of S and N status in seed and leaves. For example, under I, concentrations of N and S in leaf tissue and seed (**Table 7**) were higher in both T2 and T6 seed compared to C, possibly due to enhanced uptake and mobility of N and S resulted from S and S + N application. The consistent higher N/S ratio in 2005 and 2006 indicates that, compared to the C, the rate of increase of seed S in T2 and T6 was higher than the rate of increase in seed N in T2 and T6. For example, compared to C in 2005, the rate of increase in seed S was 5% in T2 and 40% in T6. However, the rate of increase in seed N was 22% in T2 and 20% in T6. The same general trend was noticed in 2006. The opposite trend of N/S ratio in leaf and seed indicates that the rate of leaf N increase in T2 and T6 was higher than the rate of leaf S increase. For example, the rate of increase in N in T2 was 36%, and in T6 was 44% compared to the increase of S in T2 (23%) and in T6 (17%). The dynamics of S and N status in leaves and seed indicate that there is a minimum level of both S and N required in tissues to impact seed S and N, and as a result, influencing seed protein, oleic acid, and linolenic concentrations. Therefore, the consistent higher seed protein and oleic acid in T2 and T6 than the C under I conditions indicate the significance of S or S + N application in altering seed constituents under irrigated conditions in ESPS in the Midsouth. Our results are consistent with the observation that seed protein required a high demand of mobilization of vegetative N [28,29] and S [30] to seed. Since the level of N and S concentrations in leaf could limit seed constituent concentrations, especially protein and oleic acid, maintaining N and S concentration in leaf tissue at vegetative stage is important for higher total seed protein and oleic acid, especially under I conditions.

Under NI, N/S ratio did not change in S and N treatments compared to the C because N did not change in seed, although a small percentage of seed S increase in T2 and T6 was observed. The rate of increase in leaf S was higher in T2 and T6 compared to the C, but no leaf N change was observed, reflecting that the application of N may not lead to higher N in seed under non-irrigated. This may be due to lower uptake and mobility of N under NI conditions due to water stress. The inverse relationship between oleic and linolenic acid concentrations was expected, as it has been shown in previous research [25,27], and emphasized that the increase in seed quality for protein and oleic acid occurred at the expenses of oil and linolenic acid.

Table 7. Percentage (%) of seed and leaf N and S, and N:S ratio as affected by S and N supply treatments (Treat)[†] under Irrigated (I) and Non-irrigated (NI) conditions in 2005 and 2006. Leaf refers to the fully expanded leaf at R5-R6 stages[a].

	2005			2006	Seed		2005			2006		
	I			I			NI			NI		
Treat	N	S	N:S ratio	N	S	N:S ratio	N	S	N:S ratio	N	S	N:S ratio
C	5.1	0.43	12.3	4.6	0.46	10.2	6.7	0.33	6.7	6.3	0.36	17.9
T2	6.2	0.58	10.8	5.2	0.63	8.3	6.7	0.37	6.7	6.0	0.41	14.5
T4	5.2	0.43	12.2	5.8	0.52	11.2	6.4	0.34	6.4	6.6	0.39	17.1
T6	6.1	0.60	10.3	5.6	0.57	9.9	6.6	0.38	6.6	6.5	0.40	16.4
LSD	0.21	0.03	0.72	0.35	0.03	0.70	0.12	0.020	0.12	0.27	0.022	1.08
	2005			2006	Leaf		2005			2006		
	I			I			NI			NI		
Treat	N	S	N:S ratio	N	S	N:S ratio	N	S	N:S ratio	N	S	N:S ratio
C	3.6	0.35	10.4	4.2	0.35	12.4	4.8	0.38	12.9	4.9	0.33	14.8
T2	4.9	0.43	11.6	5.2	0.41	12.7	3.9	0.34	11.5	4.3	0.35	12.2
T4	5.4	0.40	13.7	5.1	0.41	12.5	4.3	0.33	14.1	4.2	0.36	12.7
T6	5.2	0.41	12.8	5.1	0.37	14.0	3.9	0.36	11.0	4.2	0.34	13.0
LSD	0.29	0.013	0.92	0.36	0.030	1.44	0.30	0.026	1.46	0.3	0.030	1.96

[a]control (C) = 0 kg·S/ha + 0 kg·N/ha; Treatment 2 (T2) = 44.8 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S+112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha.

Table 8. Total seed N and S (kg of N or S/ha), and N:S ratio as affected by S and N supply (T1, T4, and T6) treatments† under irrigated and non-irrigated conditions in 2005 and 2006[a].

	Irrigated				Non-irrigated			
	2005		2006		2005		2006	
Treat	N	S	N	S	N	S	N	S
C	134	11.1	124	12.2	130	6.4	162	9.2
T2	173	16.2	157	19.1	122	6.9	166	11.3
T4	128	10.6	182	16.4	131	6.9	197	11.7
T6	170	16.6	158	16.4	112	6.6	177	10.8
LSD	9.7	1.1	12.1	1.62	9.6	0.71	22.2	1.48

[a]Control (C) = 0 kg·S/ha + 0 kg·N/ha; Treatment 2 (T2) = 44.8 kg·S/ha + 0 N; Treatment 4 (T4) = 0 S + 112 kg·N/ha; Treatment 6 (T6) = 44.8 S/ha + 112 kg·N/ha.

Previous research showed that protein concentration may vary, depending on environmental stress factors such as temperature [27,31] and drought [8,31,32]. It was reported that severe drought can lead to a decrease in protein concentrations [32], but others reported that severe drought increased protein concentration and content by 4.4% and 10.8%, respectively, while oil concentration and content decreased by 2.9% and 18.0%, respectively [25]. Protein and oil have been shown to exhibit a strong negative correlation (r = –0.87), indicating their inverse relationship [7,25,27,33]. It was suggested that drought during seed fill facilitates the deposition of a greater proportion of protein at the expense of oil, and 14.8% more protein and 18.3% less oil were found in seed from plants exposed to severe drought and high temperatures during seed fill [25]. On the other hand, other authors, working on soybean seed oil within genotypes differing in fatty acid profile, found that under irrigated vs. nonirrigated conditions oleic acid tended to be higher in eight of the nine genotypes, and linolenic acid was lower in six of the nine genotypes [34] and concluded that irrigation has little effect on unsaturated fatty acid content. It was showed that fatty acid percentage was consistent; however, subtle differences occurred in the profiles, concluding that irrigation did not significantly affect the fatty acid contents of MG IV or MG V [35]. Our results showed that seed protein and oleic acid increased and oil and linolenic decreased in the C compared with S or S + N supply under NI conditions. It appears that the increase of oleic acid under drought or higher temperature may suggest a possible role of this acid under environmental or chemical stresses [8,17,23, 26]. The inconsistent results in the literature of the effect of irrigation on protein and fatty acids could be due to cultivar differences [26,36,37], maturity group differences [38], or time to maturity [27]. This is because genotype × environment interactions were significant for seed yield, protein, and oil [22].

Under ESPS conditions in the Midsouth, the period from June to August coincides with initial bloom to full bloom (R1-R2) in June, beginning pod to full pod to beginning seed in July, and full seed to full maturity in August. During July to August, the critical stage of seed development (seed-fill), water deficit reached –136 mm [26]. In our experiment under non-irrigated conditions, the soil water potential reached –192, –179, –110 kPa in June, and –68, –180, and –131 kPa in August, respectively, in 2005, 2006, and 2007. Our research, using automated soil water potential sensors, indicated that about –15 kPa represents the field capacity for our soil type, and –50 to –60 kPa represents water stress conditions. Generally, after a regular irrigation (once per 7 - 10 days), soybean need about 57 mm of water to avoid water stress. This amount can increase to 76 mm, depending on the stage of the crop and soil type. Based on soil water potential and water deficit data, soybean in the NI experiment were grown under drought/water stress, especially during the critical stages, as indicated above. The rainfall in 2005, 2006, and 2007, in June, July, and August was not uniformly distributed (**Figure 2**, **Table 9**) [5,39]. Therefore, the decrease in yield under NI and alteration in seed compositions, especially concentration and total protein, oil, oleic, and linolenic acid, could be due to drought.

5. CONCLUSIONS

Sulfur or N + S application under irrigated conditions significantly increased the concentration of N and S in seed and leaf as well as seed protein and oleic acid concentrations, but decreased oil and linolenic acid concentrations, demonstrating the significant effect of S or S + N management in altering seed constituents. The increased of oleic acid under NI in the C may suggest a possible role of oleic acid as drought stress indicator. The inverse relationship between seed protein and oil, and between oleic acid and linolenic acid concentrations

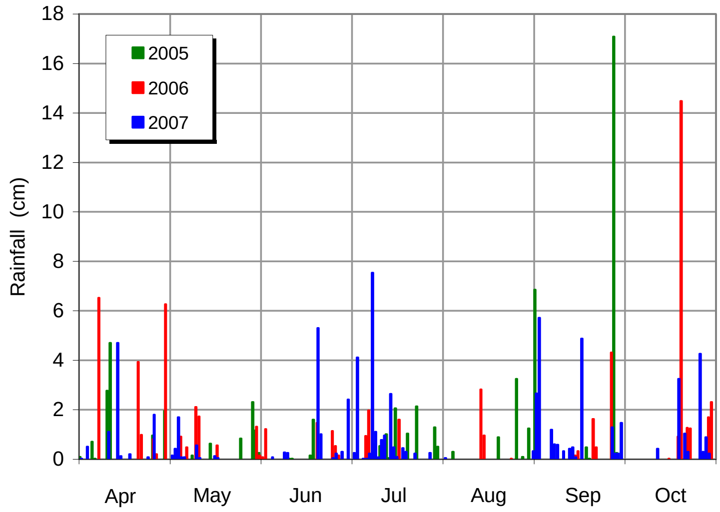

Figure 2. Rainfall (cm) in 2005, 2006, and 2007. Data were obtained by downloading Stoneville Weather Data Comparison, MS (Pringle and Ebelhar, 2009) at site http://www.deltaweather.msstate.edu/ag_weather_products/weather_comparison.htm.

Table 9. Rainfall, Pan Evaporation and Water Deficit in 2005, 2006, and 2007 for Growing Season Months[a].

Month	Rainfall, Cm			Pan Evaporation, cm			Deficit		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
Apr	11.5	18.8	8.6	19.0	17.1	17.3	–7.5	1.7	–8.7
May	5.4	7.3	3.2	20.7	18.9	21.0	–15.4	–11.6	–17.8
Jun	1.9	4.6	9.9	21.0	22.5	20.9	–19.2	–17.9	–11.0
Jul	10.6	4.5	19.7	18.2	22.0	16.5	–7.6	–17.5	3.2
Aug	12.7	4.0	8.7	19.5	22.0	21.8	–6.8	–18.0	–13.1
Sep	17.7	6.9	11.8	16.0	19.3	15.3	1.9	–12.4	–3.5
Oct	0	22.0	10.7	14.1	12.0	11.4	–14.1	10.0	–0.7

[a]Data were obtained from MSUCares. 2010. Home page. Available at http://ext.msstate.edu/anr/drec/weather.cgi (accessed February 2010; verified 4 Feb. 2010). Mississippi State Univ., Extension Services, Mississippi.

remains a challenge for soybean breeders to select for higher protein and higher oils. The observation that higher protein or oil was accompanied with higher seed and leaf tissue N and S indicates that maintaining adequate levels of N and S in leaves and seed is important for maintaining higher protein and oleic acid, especially under irrigated conditions. Further studies are needed to investigate the response of other soybean cultivars to S or S + N fertilizers. Therefore, the current response trend of the studied soybean cultivar to N or S + N application cannot be generalized.

Since commercial and public breeders are working to genetically modify soybean to produce increased oleic acid and decreased linolenic acid in the oil, the new knowledge obtained from our experiment help select target location to grow new value-added soybeans when they are released. Also, the results are beneficial for soybean industry for estimating total seed protein and oils for soybean processors under irrigated and non-irrigated conditions.

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