Soybean Seed Protein and Oil Contents and Fatty Acid Composition Adjustments by Drought and Temperature

D.L. Dornbos, Jr.*[a] and **R.E. Mullen**[b]
[a]CIBA-GEIGY Seed Division, Greensboro, NC 27419-8300 and [b]Department of Agronomy, Iowa State University, Ames, Iowa

Environmental stress during soybean [*Glycine max* (L.) Merr.] seed fill can alter the chemical composition of the seed and reduce yield, viability, and vigor. The effect of drought and high air temperature (AT) on soybean seed protein and oil contents have not been reported. The objective of this study was to characterize the protein and oil contents and fatty acid composition of soybean seeds after exposure to drought and high AT during seed fill. Experiments were conducted during two years, in which three drought-stress levels were maintained throughout seed fill. In Experiment I, "Gnome" soybeans were grown at daytime AT of 20 and 26°C, and in Experiment II "Hodgson 78" were grown at 27, 29, 33, and 35°C. Across experiments, severe drought increased protein content by 4.4 percentage points, while oil content decreased by 2.9 percentage points. As drought stress increased, measured by accumulating stress degree days, protein content increased linearly and oil content decreased linearly at each AT. Seeds from plants exposed to 35°C during seed fill contained 4.0 percentage points more protein and 2.6 percentage points less oil than those exposed to 29°C when averaged across drought stress levels. Drought had little effect on the fatty acid composition of the oil, but high AT reduced the proportion of the polyunsaturated components.

KEY WORDS: Chemical composition, environmental stress, heat and drought stress, oil quality, seed utilization.

Soybean [*Glycine max* (L.) Merr.] seed is a valuable commercial source of edible oil and protein meal and contains approximately 42% protein and 23% oil at maturity (1). The large majority of the components extracted by processors are storage compounds, the globulins stored in protein bodies and the triglycerides stored in oil bodies.

It has been understood for some time that seed oil content is positively correlated with the mean air temperature (AT) during seed fill (2–4). Soybean seeds that developed at daytime ATs of 29.4, 25.0, and 21.1°C contained oil percentages of 23.2, 20.8, and 19.5%, respectively, but neither seed yield nor protein content were affected by AT (4). Air temperatures during the 20- to 40-day period before maturity, the period during seed fill when the majority of oil is accumulated, altered oil content the most. The effect of stressfully high ATs (common in many soybean growing areas) on seed chemical composition is not understood.

Several environmental factors are responsible for changes in the proportion of unsaturated fatty acids in soybean oil. Maximum and minimum AT explained 84 and 69% of the variability in fatty acid composition (5). Linolenic acid content is reduced by high AT during seed fill (6). Soybean genotype interacted with the environment to an oil low in linolenic acid (7). Therefore, oil quality and content vary in response to AT during seed fill.

Various responses in protein and oil contents and in fatty acid composition are attributable to AT in other crop species. Linseed *(Linum usitatissimum)* oil content is negatively correlated with AT (8,9). In response to elevated ATs, sunflower *(Helianthus annuus)* oil content increased (10), decreased (11), exhibited a maximum at 21°C (5), and did not change (12). Alternatively, no relationship was identified between sunflower seed oil content and AT or solar-radiation intensity, but there was one with day length (13). Increased AT promoted the accumulation of a more saturated oil. The percentage of linoleic and linolenic acid in linseed oil decreased and oleic acid increased with AT because oleate desaturase activity is inhibited by high AT (9).

Environmental stress during soybean development can dramatically limit productivity. Soybean seed yield, seed size, and seed number can be reduced dramatically by high AT and drought stress during seed fill (14,15). Seed germination and vigor also are reduced by stress during seed fill, but to a lesser extent.

The effects of drought stress and ATs greater than 29.4°C during seed fill on soybean seed chemical composition have not been investigated under controlled environmental conditions. Concurrent changes in seed yield and chemical composition caused by environmental stress could have a dramatic and dynamic effect on both the quantity and quality of protein and oil that is extractable by seed processors. Therefore, the objective of this study was to relate changes in soybean seed yield, protein and oil contents, and fatty acid composition caused by three drought stress levels, at optimum and stressfully high ATs, in a controlled greenhouse environment during seed fill.

MATERIALS AND METHODS

Soybean plants were grown to maturity in the Agronomy greenhouse at Iowa State University (Ames, IA). Experiments I and II were both conducted in 1985 and 1986 as described previously (14,15). Adequate soil moisture was maintained until beginning seed fill both years.

In Experiment I, three drought stress treatments were imposed on "Gnome" cultivar soybeans by differential watering each day with independent trickle irrigation systems. Thirty-three pots, with one plant per pot, were spaced by 10 cm and randomly assigned to one of the three drought stress treatments. The volume of water required to saturate the control (unstressed) pots was delivered and measured daily. Seventy-five and fifty percent of the volume required for saturation of the control pots was delivered to the remaining plants to impose and maintain the moderate and severe drought-stress treatments, respectively. Drought-stress intensity was measured by accumulating stress degree days (SDD) during seed fill. SDD were determined by summing the average daily leaf temperature and AT difference (16) during seed

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*To whom correspondence should be addressed at CIBA-GEIGY Seed Division, P.O. Box 18300, Greensboro, NC 27419-8300.

fill as described earlier (14). Day/night ATs of 20/16 and 26/16°C were maintained in 1985 and 1986, respectively, as measured daily with a shielded hygrothermograph.

In Experiment II, half of 144 pots were placed in one of two adjacent similar greenhouse rooms after the beginning of seed fill. The AT in each room was measured continuously during seed fill with a shielded hygrothermograph. Day/night ATs of 29/20 and 35/20°C were maintained (with 16/8 hr day/night period) throughout seed fill during 1985, and ATs of 27/20 and 33/20°C were maintained during seed fill in 1986. Twenty-four of the seventy-two plants in each room were randomly chosen to receive either the control, moderate, or severe drought stress treatment. Drought stress levels were administered and measured in each greenhouse room as in Experiment I and as described previously (15).

When mature, the seeds from each plant were hand-harvested and bulked with the seed from the other plants that received the same AT and drought stress treatment. Five- to seven-gram seed samples were randomly withdrawn from each bulk for chemical analyses. The seed material was dried and uniformly ground for measurement of protein and oil content by infrared reflectance spectroscopy at the USDA/ARS/NCAUR (Peoria, IL).

Fatty acid composition of the total seed oil from 3 5-seed samples randomly sampled from each treatment was determined by gas chromatography. The seed samples were dried in a vacuum oven at 0.7 MPa and 85°C for approximately 15 hr, then crushed with a hydraulic press at 1055 kg/cm. The total oil was extracted by soaking the seed material in 3 mL of distilled hexane for 48 hr; then 0.2 mL of the extract was placed in a 2-mL glass vial. Fatty acid methyl esters were prepared by addition of 0.5 mL of 1 M sodium methoxide in methanol and allowed to react for 1.5 hr. The transesterification reaction was stopped by addition of distilled water. The esters were partitioned from the reaction mixture by addition of 1 mL hexane. Approximately 1 hr was allowed for the esters to partition into the hexane layer. Approximately 1.5 mg of fatty acid esters in hexane were separated on a Beckman GC-5 gas chromatograph equipped with a 2 m × 3.2 mm I.D. glass column packed with 15% EGSSX on Chromosorb W (100/120 mesh) at 185°C. Nitrogen gas (40 mL/min) was used as the carrier. Fatty acids were detected by flame ionization with hydrogen gas and flow rates of 50 and 300 mL/min, respectively. Fatty acid ester retention times were identified by comparison with authentic standards.

Treatment effects were considered significant when $P \leqslant 0.05$, as determined by analysis of variance and regression techniques.

RESULTS AND DISCUSSION

Environmental stress imposed in these experiments was sufficiently severe to reduce soybean seed yield, dramatically in some cases. In Experiment I, moderate and severe drought stress reduced the seed yield of "Gnome" soybeans by 30 and 38% in 1985, respectively, and by 23 and 58% in 1986 (14). In Experiment II, "Hodgson 78" seed yield was reduced 47% by severe drought at 29°C and 64% at 35°C in 1985 (15). In 1986 seed yield was reduced 38 and 42% by severe drought at 27 and 33°C, respectively. Soybean seed yields were reduced considerably by drought stress in both experiments, and to a much greater extent when drought occurred in conjunction with high-temperature stress in Experiment II.

The extent to which drought can affect the chemical composition of the soybean seed has not been reported. Protein and oil content of seed from "Gnome" soybeans were strongly affected by drought stress during seed fill (Table 1). Seed from severely drought-stressed plants contained 10.2% and 11.8% more protein in 1985 and 1986, respectively. Inversely, severely drought-stressed seed contained 9.9% and 12.4% less oil. Drought stress during seed fill altered the chemical composition of the soybean seed similarly both years.

A second experiment was conducted in controlled chambers with "Hodgson 78" soybeans to investigate the combined effect of drought and high-temperature stress on protein and oil content. In 1985 at 29°C and in 1986 at 27°C, near the optimum daytime AT for photosynthesis and growth of soybean, protein content increased as the intensity of drought stress increased (Table 2). Soybeans

TABLE 1

Protein and Oil Content of Seed from Soybean (cv. "Gnome") Plants Exposed to Drought Stress During Seed Fill[a]

Year	Drought stress level	Protein (%)	Oil (%)
1985	Control	40.3	21.3
	Moderate	42.3	20.3
	Severe	44.9	19.2
	LSD (0.05)	0.6	0.9
1986	Control	37.4	23.3
	Moderate	39.5	22.2
	Severe	42.4	20.4
	LSD (0.05)	1.5	1.4

[a] Day/night temperatures were maintained at 20 and 16°C, respectively, in 1985; and at 26 and 16°C, respectively, in 1986.

TABLE 2

Protein and Oil Content of Seed from Soybean (cv. "Hodgson 78") Plants Exposed to Drought and High-Temperature Stress During Seed Fill

Year	Temp. (°C)	Drought stress level	Protein (%)	Oil (%)
1985	29	Control	38.7	24.3
		Moderate	39.2	23.5
		Severe	43.7	21.8
	35	Control	41.5	23.5
		Moderate	45.2	20.8
		Severe	47.0	17.6
		LSD (0.05)	0.9	0.7
1986	27	Control	37.3	24.9
		Moderate	37.4	25.1
		Severe	39.3	23.7
	33	Control	38.1	25.4
		Moderate	39.8	24.4
		Severe	42.2	22.6
		LSD (0.05)	1.3	1.2

that developed under severe drought stress contained 11.4% more protein than seeds from control plants in 1985 and 5.1% more protein in 1986. Drought reduced the oil content of the seeds by 10.3 and 4.8% in 1985 and 1986, respectively.

Increased drought stress intensity had the same effect at the high AT levels of 35°C in 1985 and 33°C in 1986. When averaged across years, severe drought increased the protein content of the seed by 10.8% and decreased the oil content by 18.0% (Table 2). Both years, protein and oil exhibited a strong negative correlation ($r = -0.87^{**}$), bearing out their inverse relationship. The data from Experiments I and II suggest that drought during seed fill facilitates the deposition of a greater proportion of protein at the expense of oil, regardless of the genetic constitution of the two varieties or ATs tested.

Drought stress intensity was quantified by accumulating SDD as described previously (15,16). When protein and oil content were regressed against SDD, linear relationships were significant for both varieties and years tested (Table 3). Therefore, as the intensity of drought stress increased during seed fill, the protein content of the seed subsequently produced increased linearly and the oil content decreased linearly. Because SDDs can be as readily measured in the field as in controlled growth chambers, particularly in less humid areas, the effect of drought stress on soybean's chemical composition can be readily studied in the production environments of different geographical areas.

Air temperature during seed fill also impacted the chemical composition of the soybean. Control (well-watered) plants grown at an AT of 35°C during seed fill produced seeds with 6.7% more protein and 3.3% less oil than those grown at 29°C in 1985 (Table 2). In 1986, plants grown at an AT of 33°C produced seeds with 2.1% more protein and 2.0% more oil than those grown at 27°C. Differences in seed protein and oil content were similar, but larger, due to the elevated AT during seed fill when plants were grown under severe drought stress. When averaged across years, 14.8% more protein and 18.3% less oil were contained in seeds from plants exposed to severe drought and high temperatures during seed fill.

At first glance, the effects of AT on soybean seed composition seem to conflict with earlier studies, in which oil content was found to increase with AT, whereas protein content was unaffected (3,17). Soybean seed oil percentage was positively correlated with AT for five Maturity Group 0 varieties grown for one to three years at eight locations, and for six Group VIII varieties grown for two or three years at nine locations (3). In growth chambers, seed oil content increased linearly 15.9% when daytime maximum AT increased from 21.1 to 29.4°C, but yield, protein, and non-protein nitrogen were not affected (4). The maximum AT of the previous studies corresponded closely with the minimum AT of the results reported here with "Hodgson 78" (Table 2). Therefore, these data represent a higher temperature range than reported before and suggest the possibility of a non-linear effect of AT on protein and oil content.

Protein and oil content data from the study with "Gnome" soybeans (grown at daytime ATs of 20°C in 1985 and 26°C in 1986), taken together with "Hodgson 78" data, suggest an inverse curvilinear relationship between protein, oil, and AT during seed fill. Protein content declined between ATs of 21 and 27°C, then increased. Oil content increased between ATs of 21 and 29°C, then decreased. These data demonstrate that AT during soybean seed fill does alter the chemical composition of the seed produced, and that a critical temperature may exist near 28°C for maximum oil and minimum protein content, similar to that reported for sunflower oil (5).

The changes in protein and oil contents as the result of environmental stress are associated with large reductions in seed yield (Table 4). Because protein content increases with drought and high AT, while that of oil decreases, the protein/oil ratio increases dramatically from 1.55 in seed from an unstressed environment to 2.31 in seed that developed under severe drought and high ATs.

TABLE 3

Regression Equations, R^2, and Significance for the Relationship Between Soybean Seed Protein and Oil Content, and SDD During Seed Fill

Cultivar	Air temp. (°C)	Independent variable	Slope	Intercept	R^2
1985					
"Gnome"	20	Protein	0.063	36.9	0.65**
	20	Oil	−0.028	22.6	0.66**
"Hodgson 78"	29	Protein	0.089	32.6	0.92**
	35	Protein	0.067	38.4	0.89**
	29	Oil	−0.043	27.1	0.83**
	35	Oil	−0.072	27.2	0.97**
1986					
"Gnome"	26	Protein	0.050	34.8	0.87**
	26	Oil	−0.028	24.8	0.76**
"Hodgson 78"	27	Protein	0.033	34.9	0.69**
	33	Protein	0.073	35.0	0.79**
	27	Oil	−0.021	26.5	0.62*
	33	Oil	−0.050	27.6	0.70**

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

TABLE 4

Protein and Oil Quantities Available to Soybean Seed Processors Following Exposure to Drought and High-Temperature Stress During Seed Fill

Year	Temp. (°C)	Drought stress level	Yield	Protein	Oil	Protein/Oil
				(g/plant)		
1985	29	Control	34.3	13.3	8.3	1.60
		Moderate	24.0	9.4	5.6	1.68
		Severe	18.3	8.0	4.0	2.00
	35	Control	24.4	10.1	5.7	1.77
		Moderate	14.8	6.7	3.1	2.16
		Severe	8.7	4.1	1.5	2.73
1986	27	Control	38.0	14.2	9.5	1.49
		Moderate	31.4	11.7	7.9	1.48
		Severe	23.7	9.3	5.6	1.66
	33	Control	29.4	11.2	7.5	1.49
		Moderate	26.4	10.5	6.4	1.64
		Severe	17.0	7.2	3.8	1.89

But, because less seed is produced in the stressful environments, the quantity of both protein and oil available to processors is dramatically reduced. Across years, the quantity of protein in the seed from each plant was reduced 29% by the high-AT treatment and 42% by severe drought stress (Table 4). Oil quantities were reduced further—35% by high AT and 53% by severe drought stress. Even though the proportion of protein increases with stress, the quantity available to processors is less as a function of reduced yield.

Drought stress during seed fill had only a small effect on the fatty acid composition of the seed oil, unlike protein and oil contents. Drought stress increased the proportion of stearic acid in seed from "Hodgson 78" in 1985, while oleic acid content decreased in 1986 (Table 5). Earlier studies with "Gnome" suggested that drought during seed fill altered fatty acid composition of the oil slightly, but inconsistently (data not shown).

High AT during soybean seed fill proportionately reduced the percentage of linoleic and linolenic acids, and increased that of oleic acid (6,17). In 1985, the high AT during seed fill reduced the proportion of linoleic and linolenic acid in the soybean seed oil by the sum of 11.1% while oleic acid compensated by increasing 10.0% (Table 5). Similarly, oleic acid content increased by 12.3% in 1986 because of the high AT during seed fill, again compensating largely for the 11.4% decrease in linoleic and linolenic acid. A similar relationship between high AT, oleic, linoleic, and linolenic acid was reported with linseed oil (9). In linseed, the high AT reduced the activity of oleic acid desaturase, and the proportion of linoleic and linolenic acid in the oil (9). Because linoleic and linolenic acid are synthesized by the consecutive desaturation of oleic acid in developing soybean seeds (18), oleic acid desaturase activity may also have been inhibited by high AT during seed fill in our study, giving rise to increased quantities of oleic acid and reduced quantitites of linoleic and linolenic acid. Correlation coefficients of −0.59**, −0.83**, and 0.66** between linolenic acid and stearic, oleic, and linoleic acid, respectively, in this study concur with the purported activity of oleic acid desaturase.

In summary, both drought and high AT stress during soybean seed fill affect the proportion, quantity, and quality of the seed oil and protein available to processors. Understanding the environmental conditions of the production environment may help processors predict the quantity and quality of the desired products obtainable from the soybean seed.

TABLE 5

Fatty Acid Composition of "Hodgson 78" Soybean Seed Oil After Exposure to Drought and High-AT Stress During Seed Fill

Year	Temp. level	Drought stress level	Fatty acid (%)				
			16:0	18:0	18:1	18:2	18:3
1985	29	Control	11.9	3.5	24.2	53.3	7.1
		Moderate	12.1	3.6	23.1	53.8	7.4
		Severe	12.0	3.4	23.3	53.8	7.4
	35	Control	11.7	4.3	26.9	51.2	5.8
		Moderate	11.9	4.6	27.7	49.7	6.1
		Severe	12.2	4.6	28.9	48.5	5.9
		LSD (0.05)	0.4	0.2	1.2	1.0	0.4
1986	27	Control	11.7	3.6	24.9	53.2	6.6
		Moderate	11.5	3.4	26.1	52.6	6.4
		Severe	11.6	3.5	27.0	51.5	6.5
	33	Control	11.2	3.7	28.4	51.5	5.3
		Moderate	11.1	3.7	29.0	51.1	5.1
		Severe	11.0	3.8	29.6	50.2	5.4
		LSD (0.05)	0.2	0.1	1.7	1.3	0.5

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