Phospholipids of Environmentally Stressed Soybean Seeds[1]

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Protein and oil content of the soybean [*Glycine max* (L.) Merr.] seed and the fatty acid composition of the oil can be altered by environmental stress. The objective of this study was to characterize the composition of the phospholipid (PL) from soybean seeds after exposure to drought and high temperature during seed fill. Drought stress was imposed on greenhouse-grown soybean plants at temperatures of 28 and 33°C after the beginning of seed fill and was maintained throughout the seed-fill period. The fatty acid composition of each PL class was altered by drought and high temperature. With phosphatidylcholine and phosphatidylethanolamine, which composed 89% of the separated PL, greater proportions of 16:0 and 18:0 and lesser proportions of 18:2 and 18:3 were present in soybean seeds exposed to high temperature and severe drought. More linolenic acid and less palmitic acid were present in phosphatidylinositol. The changes were comparable to those of the triglyceride because of high temperature. The elevated temperature increased the proportion of phosphatidylcholine and phosphatidylinositol and decreased that of phosphatidylethanolamine. The effect of drought and high temperature stress on PL class and fatty acid composition has important implications on the quality of soybean seed oil and lecithin and on the ability of the seed to maintain optimum rates of metabolism in the development and germination environment.

Soybean [*Glycine max* (L.) Merr.] is a valuable seed crop produced for its oil, protein (meal), and lecithin. Drought and high-temperature stress during seed fill can reduce the yield, germination, and vigor of the seed (1). Protein and oil content exhibit an inverse curvilinear relationship between the temperatures of 20° and 35°C (2). The oil content of seeds from well-watered plants was maximum (24.6%), and protein content was minimum (38%), at approximately 29°C. Protein content increased, and oil content decreased, linearly when drought-stress intensity increased. High temperature during seed fill decreased the content of 18:2 and 18:3 in the total soybean oil and increased that of 18:1 (2). Drought had little effect on fatty acid composition (2).

The phospholipids (PL) make up 1.5 to 5.0% of the crude hexane extractables from the soybean seed (3). Soybean seed PL is made up of three major classes: 35 to 46% phosphatidylcholine (PC), 25 to 27% phosphatidylethanolamine (PE), and 13 to 18% phosphatidylinositol (PI) (4,5,6). Each PL class has a characteristic fatty acid composition. Phosphatidylcholine contains between 14.9 and 20.5% palmitic acid (16:0), 3.2 and 6.3% stearic acid (18:0), 7.5 and 13.7% oleic acid (18:1), 58.8 and 64.8% linoleic acid (18:2), and 2.0 and 6.0% linolenic acid (18:3) (7). Phosphatidylethanolamine contains between 15.8 and 31.6%, 3.2 and 4.4%, 8.4 and 8.7%, 53.2 and 64.7%, and 3.2 and 6.3% of 16:0, 18:0, 18:1, 18:2, and 18:3, respectively, and PI contains between 25.6 and 47.7%, 8.2 and 11.7%, 4.9 and 9.1%, 36.2 and 44.4%, and 2.7 and 6.4% (7).

The PL composition of microorganisms is altered by changes in temperature. When *Tetrahymena pyriformis* was transferred from 39.5° to 15.0°C, 18:2 and 18:3 percentages increased, and 16:0 decreased, within 30 min (8). When the temperature decreased from 35° to 20°C, decreased membrane fluidity stimulated desaturase activity, causing the irreversible desaturation of fatty acids in *Bacillus licheniformis* and a shift in the saturation:unsaturation ratio from 20:1 to 2:1 (9). In *Fusarium oxysporum*, the PE:PC ratio decreased when the temperature increased from 15° to 37°C (10). When the growth temperature is reduced, optimum membrane fluidity and functionality are maintained at the new temperature by changes in PL class and fatty acid composition (11).

The PL content of chilling-tolerant plant species doubled and the rate of fatty acid desaturation increased when the temperature decreased during vegetative growth (12,13). In chilling-sensitive plants, growth ceased between 0° and 12°C while chilling-tolerant plants continued to grow (14). The membrane-lipid composition of cold-tolerant plants is adjusted in response to chilling temperatures, maintaining fluid membranes, metabolism, and growth (15). Changes in seed PL composition resulting from exposure to high temperature or drought stress have not been described previously. Therefore, the objective of this study was to characterize the changes in soybean seed PL composition that resulted from exposure to drought and high-temperature stress during soybean seed fill.

MATERIALS AND METHODS

Soybean (cultivar "Hodgson 78") plants, 1 per pot, were grown to maturity in the Agronomy greenhouse at Iowa State University, Ames, Iowa, as described earlier (1,2). Adequate soil moisture and fertility were maintained until beginning seed fill. Half of the plants were then maintained at a constant maximum temperature of 27°C for 16-h day's throughout seed fill. The remaining plants were maintained at a constant maximum temperature of 33°C for 16-h day's. Night temperatures were maintained at a constant 19°C throughout seed fill. Three drought stress treatments were imposed independently by differential watering each day with independent trickle irrigation systems. The volume of water required to saturate the well-watered (control) pots was delivered and measured daily with a trickle irrigation system. Seventy-five and fifty percent of the volume required for saturation of the control pots were delivered to the remaining plants to impose and maintain the moderate and severe drought-stress treatments, respectively. When mature, the seeds from each plant were hand-harvested and bulked, and random samples were withdrawn from the bulks for chemical analysis.

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Between 5 and 6 g (fresh weight, 8–10% moisture) of seed was finely grown with a Wiley mill. The lipid was extracted for 1.5 h with 275 ml of 1:1:0.75 (by vol) methanol:chloroform:water and 0.05% (w/v) butylated hydroxytoluene and the residue was washed with 50 ml of 2:1 (v/v) chloroform:methanol (16). The chloroform layer of the combined filtrates was dried under nitrogen and weighed. The extraction efficiency of the oil was approximately 94% when compared with its measurement by infrared reflectance.

A SPICE^R (Analtech, Inc.) cartridge was used to separate the PL from the lipid extract (17), which was dissolved in approximately 2.5 ml of 100:1 (v/v) chloroform:acetic acid. Tri- and di-glycerides were eluted with 10 ml 100:1 (v/v) chloroform:acetic acid and then the PL with 5 ml of 100:50:40 methanol:chloroform:water (by vol). Ten milliliters of water and 7.5 ml of chloroform were added to the combined PL fractions and the lipid-containing layer was dried under nitrogen and weighed.

PI, PC, and PE were isolated from PL on silica gel G TLC plates (J.T. Baker) with 80:15:5:2 (by vol) chloroform:methanol:acetic acid:water (6). Average Rf values were 0.14 for PI, 0.26 for PC, and 0.56 for PE. The silica corresponding to PI, PC, and PE was extracted twice with 6 ml 2:1 (v/v) chloroform:methanol and reduced to dryness under nitrogen.

Fatty acid methyl esters (FAME) were prepared after saponification and esterification with BF_3 in methanol. The FAME were extracted into hexane and analyzed on a Beckman GC-5 gas chromatograph with 15% EGSSX on Chromosorb W. Nitrogen was the carrier and detection was by flame ionization.

RESULTS AND DISCUSSION

Larger amounts of PL were isolated from the oil of soybean seeds exposed to environmental stress during seed fill than from those exposed to optimum growth conditions (Table 1). Environmental stress also reduced the reproductive period duration from 39 to 22 days (2). The percentage of PL in the oil has been reported to decrease steadily during seed fill from 49.0 to 3.8% between 9 and 80 days after flowering (5). A greater proportion of the oil may have been PL in this study because environmental stress shortened the seed-filling process, possibly during a period of rapid triglyceride biosynthesis.

The relative proportions of the three PL classes changed in response to both temperature and drought during seed fill (Table 1). High temperature increased the proportion of PC and PI and decreased that of PE. The presence of less PE in the membrane represents an adaptation to high temperature, minimizing the propensity for the amino group of adjacent PE molecules to form a deleterious covalent bond upon dehydration or heating (J.H. Crowe, Dept. of Biochem., University of California-Davis, personal communication). Drought increased the proportion of PC at both temperatures, but reduced PE only at 27°C and, PI at 33°C. Phosphatidylcholine and PE were present in nearly equal proportions and constituted 89% of the three PL classes from the soybean oil in this study. The proportion of PE in this study is greater than that typically reported in soybean PL (18), possibly because of varietal variation.

TABLE 1

Phospholipid Content of Extracted Oil and PL Composition From Soybean Seed Exposed to Drought and High-temperature Stress During Seed Fill

Air temperature	Drought stress level	Phospholipid content of oil	Phospholipid class[a]		
			PC	PE	PI
(C)			(%)		
27	Control	1.9	29.3	66.4	4.3
	Moderate	1.8	40.8	51.4	7.7
	Severe	4.3	45.4	48.3	6.4
	Mean	2.7	38.8	54.8	6.4
33	Control	5.7	42.5	44.2	13.3
	Moderate	3.6	38.8	38.1	23.0
	Severe	4.2	47.0	46.5	6.5
	Mean	4.5	43.9	44.3	11.8
SE[b]		0.6	5.1	5.1	1.2
SE[c]		1.0	4.7	4.6	1.9

[a]Percent individual PL of the total PL. Calculated from the percent weight of fatty acid from the individual and total PL.
[b]Standard errors for comparison of temperature means.
[c]Standard errors for comparison of drought stress means.

The fatty acid content of each PL class was altered by temperature during seed fill (Table 2). With PC, larger proportions of 18:0 and 18:1 and smaller proportions of 18:2 and 18:3 were present in the seed after exposure to the high temperature. The content of PE 16:0 and 18:0 increased with temperature, whereas that of 18:2 and 18:3 decreased. With the exception of an increase in 18:3 content, the fatty acid composition of PI was unaffected by the higher temperature. The saturation:unsaturation ratio of PC and PE, which represented 89% of the soybean seed PL, increased in response to seed development at the elevated temperature. Elevated temperature during seed fill had a similar effect on the fatty acid composition of the total oil, in which the proportion of 18:2 and 18:3 increased and that of 18:1 decreased (2). The activity of the enzyme oleic acid desaturase from flaxseed oil was inhibited by the high temperature during development and contributed to the reduction in the proportion of polyunsaturated fatty acids in flaxseed oil (19). The decrease in the proportion of unsaturated fatty acids in the total soybean oil and PL resulting from exposure to high temperature may also be due to the activity of a similar enzyme.

The fatty acid composition of the PL was also altered by drought (Table 3). Again, the fatty acids of PC and PE demonstrated a greater propensity for adjustment because of stress than did those of PI. The proportion of PC 18:0 and 18:1 was increased by drought, and the proportion of 18:2 and 18:3 decreased. With PE, the proportion of 18:0 increased, whereas that of 18:3 decreased. Finally, the content of 16:0 from PI was reduced by drought. In contrast to the PL, the fatty acid composition of the total soybean oil was unaffected by drought (2). The effect of drought on the fatty acid composition of each PL class was consistent with the effect of temperature because the proportion of saturated fatty acids increased because of exposure

TABLE 2

Phospholipid Fatty Acid Composition From Soybean Seed Exposed to Optimum and Stressfully High Air Temperatures During Seed Fill

Phospholipids class	Air temperature	Fatty acid[a]				
		16:0	18:0	18:1	18:2	18:3
	(C)	(%)				
PC	27	18.5	4.8	14.3	58.5	3.9
	33	18.7	7.6	18.0	54.1	1.6
	SE	1.7	0.7	3.3	4.1	0.7
PE	27	24.0	4.1	13.0	55.4	3.5
	33	28.9	5.6	13.7	49.7	2.1
	SE	0.9	0.6	1.6	2.4	0.5
PI	27	38.2	11.8	13.6	36.4	1.8
	33	35.2	11.2	15.8	35.5	2.2
	SE	4.4	2.0	2.6	4.6	0.3

[a]Percent individual fatty acid of the total PL.

TABLE 3

Phospholipid Fatty Acid Composition From Soybean Seed Exposed to Two Levels of Drought Stress Across Temperatures During Seed Fill

Phospholipid class	Drought stress level	Fatty acid[a]				
		16:0	18:0	18:1	18:2	18:3
		(%)				
PC	Control	19.5	5.1	13.8	58.6	3.0
	Moderate	19.0	5.8	15.1	57.0	3.2
	Severe	17.4	7.8	19.4	53.4	2.1
	SE	1.5	1.3	3.5	3.6	0.9
PE	Control	23.6	3.6	14.0	55.0	3.9
	Moderate	28.5	6.3	14.0	49.0	2.3
	Severe	26.2	4.2	11.9	55.3	2.4
	SE	2.0	0.4	1.9	2.0	0.6
PI	Control	38.3	11.3	13.6	38.2	1.5
	Moderate	36.0	11.4	15.6	34.4	2.6
	Severe	36.3	11.8	14.4	35.7	1.8
	SE	1.5	1.2	1.4	3.1	0.2

[a]Percent individual fatty acid of the total fatty acid.

to drought during seed fill. The statistical interaction between drought and temperature was insignificant for each of the fatty acid components measured. Therefore, the effect of drought was similar for each fatty acid under both temperature conditions.

The changes in PL class and the fatty acid composition of each class because of a high-temperature stress were opposite to, and therefore consistent with, those reported for microorganisms and plants after exposure to a low-temperature stress. It has been demonstrated with microorganisms that adjustment in PL composition permits the maintenance of membrane fluidity and, therefore, the barrier function and metabolism of each cell (11). Because polyunsaturated fatty acids have a relatively low melting point, membranes containing a high proportion of 18:2 and 18:3 are too fluid at elevated growth temperatures to maintain the essential chemiosmotic gradients. The deposition of a larger proportion of saturated fatty acids in PC and PE after exposure to an increased temperature (Table 2), in contrast, represents an adjustment to maintain optimum membrane fluidity. Although representing an advantage for the developing seed at the high air temperature, the adjusted PL composition may represent a disadvantage in a typical germination environment in which the seedbed is relatively cool and wet. During imbibition, the membranes of seeds adapted to the high air temperature may exhibit excessive permeability to cell solutes relative to adapted membrane lipids and contribute to reduced seed vigor (20).

Two commercial soybean lecithin samples contained 82% of PC, PE, and PI (21). One emulsification property of soybean lecithin—its swelling behavior—was altered by changes in the concentration of PC, PE, PI, and phosphatidic acid (22). Changes in PL composition because of environmental stress could affect this and other physical properties of the lecithin and its potential for utilization.

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