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Changes in Soybean (*Glycine max* [L.] Merr.) Glycerolipids in Response to Water Stress[1]

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ABSTRACT

Soybean (*Glycine max* [L.] Merr.) plants with the first trifoliate leaf fully expanded were exposed to 4 and 8 days of water stress. Leaf water potentials dropped from −0.6 megapascal to −1.7 megapascals after 4 days of stress; then to −3.1 megapascals after 8 days without water. All of the plants recovered when rewatered. The effects of short-term drought stress on triacylglycerol, diacylglycerol, phospholipid, and galactolipid metabolism in the first trifoliate leaves was determined. Leaf triacylglycerol and diacylglycerol content increased 2-fold during the first 4 days of stress and returned to control levels 3 days after rewatering. The polar lipid fraction, which contained phospholipids and galactolipids, changed little during this time. The linolenic acid (18:3) content of the triacylglycerol and diacylglycerol increased 25% during stress and the polar lipid 18:3 content decreased 15%. The pattern of glycerolipid labeling, after applying [2-^{14}C]acetate to intact leaves was altered by water stress. After 4 days of water stress the radioactivity of phosphatidic acid + phosphatidylinositol, phosphatidylcholine, triacylglycerol, and diacylglycerol increased between 4 and 9% (compared to control plans) while radioactivity of phosphatidylethanolamine, monogalactosyldiglyceride, and digalactosyldiglyceride decreased 2 to 11%. These data indicated that increased levels of triacylglycerol and diacylglycerol observed during water stress were attributed to *de novo* synthesis rather than breakdown or reutilization of existing glycerolipids and fatty acids.

Water deficits have a marked effect on leaf glycerolipid composition. In maize seedlings, leaf TG[2] content doubled and fatty acid composition changed in response to a PEG induced osmotic stress of −1.5 MPa (8). The composition change in the maize seedlings involved a doubling of the linolenic acid content. In soybean, TG content increased 3-fold more in a drought susceptible line (17) compared to a tolerant genotype. The authors suggested the significance of drought induced increase in TG content of maize and soybean was to provide energy storage which made no demand on available water or was an indication of the capability for basal metabolic activity during drought. Little other information is available concerning the influence of water deficits on synthesis and pool sizes of these lipids in leaf tissue.

Chloroplast membrane structure is another aspect of lipid metabolism which is involved in drought tolerance. Chloroplast lamellae are composed of 40 to 60% MGD, 20 to 30% DGD, 5 to 10% sulfoquinovosyl diglyceride, 3 to 10% PC, and 8 to 12% PG (13). Evidence from cotton, barley, and wheat chloroplasts indicates MGD and DGD amounts decrease during water deficits (6, 7, 10). Additionally, EM work with sunflower indicates a thinning of the chloroplast membranes in response to water deficits (9). However, information is unavailable concerning the influence of water stress on soybean MGD or DGD and how they interrelate with TG, DG, and phospholipid metabolism.

In this paper we have documented changes which occurred in soybean leaf glycerolipid metabolism during a short-term water deficit. In addition, we have demonstrated a shift in the labeling pattern of glycerolipids which suggests altered patterns of biosynthesis.

MATERIALS AND METHODS

Plant Growth. Soybean (*Glycine max* [L.] Merr.) cv Williams seed was sown, 20 per 40 × 30 cm flat, into coarse sand. The plants were grown for 21 d in a growth chamber with a light intensity of 450 μE m^{-2} s^{-1} (400–700 μm), in day/night temperatures of 28°C day 23°C night and a photoperiod of 14 h. The flats were watered daily with nutrient solution as described elsewhere (12). Plants with a fully expanded first trifoliate were used for analysis. Water stress was imposed by witholding water. All experiments were replicated three times, two samples per replicate.

Water Potential. Leaf water potentials were measured with a thermocouple psychrometer using isopiestic technique (2). The psychrometer chambers were coated with melted and resolidified petrolatum (3) and measurements were corrected for the heat of respiration (1). All leaf samples were sealed with the psychrometer chambers within 10 s of excision.

Lipid Extraction. Leaf samples from the first trifoliate were weighed then frozen with liquid N_2 and freeze dried overnight. Two samples of 1.0 g dry weight from each treatment were homogenized with a Polytron[3] for 30 s at 4°C in 20 ml $CHCl_3$/

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[2] Abbreviations: TG, triacylglycerol; DG, diacylglycerol; DGD, diagalactosyldiglyceride; MGD, monogalactosyldiglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

[3] Mention of a trade name does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Table I. *Fatty Acid Composition and Amounts of Total Polar Lipid (PA, PI, PS, PC, PE, MGD, and DGD), DG and TG Fractions from Soybean Leaves after Various Periods of Water Stress*

Data are the means of three replicates, 2 samples per replicate.

Treatment	Leaf Water Potential	Total Polar Lipid Fatty Acid						Total	
		16:0	16:1	18:0	18:1	18:2	18:3		
	MPa	*mol %*						*μmol·g dry wt*$^{-1}$	*% of total*
4 d control	−0.6	11.9	3.9	3.3	2.0	7.3	71.6	42.1	94.8
4 d stress	−1.7	10.8	1.7	2.7	1.3	4.7	78.8	40.4	87.1
4 d stress rewatered	−0.8	15.1	2.4	3.4	2.0	7.8	69.3	42.9	93.0
8 d control	−0.6	12.0	3.3	3.7	2.3	7.8	69.9	44.0	93.8
8 d stress	−3.1	25.6	4.6	5.1	3.0	7.4	54.3	38.3	85.1
LSD 0.05		0.4	0.1	0.2	0.1	0.3	2.3	2.5	
		Diacylglycerol							
4 d control	−0.6	22.8	6.3	7.1	5.7	13.9	44.2	1.1	2.5
4 d stress	−1.7	13.9	4.1	3.6	2.6	8.4	67.4	2.6	5.6
4 d rewatered	−0.8	33.9	7.5	8.1	5.5	14.1	30.9	1.4	3.0
8 d control	−0.6	26.4	9.8	7.4	4.0	13.1	39.3	1.7	3.6
8 d stress	−3.1	23.4	5.0	4.9	4.7	12.2	49.8	2.5	5.6
LSD 0.05		0.6	0.2	0.3	0.2	0.5	2.5	0.8	
		Triacylglycerol							
4 d control	−0.6	18.5	4.6	6.5	9.0	18.9	42.5	1.2	2.7
4 d stress	−1.7	9.5	1.4	2.4	3.2	16.0	67.5	3.4	7.3
4 d rewatered	−0.8	17.9	1.8	6.4	5.0	17.4	51.5	1.8	3.9
8 d control	−0.6	22.5	1.8	10.4	6.9	16.8	41.6	1.2	2.6
8 d stress	−3.1	8.8	1.9	2.7	3.4	14.0	69.2	4.2	9.3
LSD 0.05		0.5	0.1	0.3	0.2	0.5	2.8	0.7	

Table II. *Fatty Acid Composition and Amounts of Various Polar Glycerolipids (GL) from Soybean Leaves which Were Either Well-Watered or Had Been Stressed for 4 Days*

Data are the means of three replicates, two samples per replicate.

Treatment	Leaf Water Potential	GL	Fatty Acid						Total Lipid
			16:0	16:1	18:0	18:1	18:2	18:3	
	MPa		*mol %*						*μmol·g dry wt*$^{-1}$
Control	−0.5	PA + PI	23.5	5.8	6.5	7.0	25.7	31.5	0.76
Stress	−1.8		26.4	10.6	7.5	5.5	22.9	27.1	2.94
Control	−0.5	PC	34.9	3.4	5.8	4.9	12.0	39.0	1.13
Stress	−1.8		32.6	4.9	6.4	3.6	16.6	35.9	2.02
Control	−0.5	PE	48.0	3.7	7.9	4.4	6.1	29.9	5.38
Stress	−1.8		53.2	1.5	6.8	4.0	5.2	29.3	3.65
Control	−0.5	PG	15.6	3.3	4.8	5.7	12.6	58.0	1.00
Stress	−1.8		21.7	3.7	8.9	11.8	9.1	44.8	1.05
Control	−0.5	DGD	23.3	3.0	7.0	2.6	5.4	58.7	18.77
Stress	−1.8		24.0	1.5	7.2	2.3	5.6	59.4	18.31
Control	−0.5	MGD	3.8	0.5	1.2	1.4	4.4	88.7	9.20
Stress	−1.8		4.2	0.5	1.8	1.6	6.2	85.7	8.23
LSD 0.05			3.5	0.1	0.2	0.3	0.8	3.8	1.20

methanol (2:1, v/v). The homogenate was filtered on Whatman No. 1 paper. The debris was removed from the paper and homogenized as before in 20 ml $CHCl_3$/methanol (2:1, v/v). The polytron and grinding vessel were washed with 15 ml methanol which was added to the filtrates. To this mixture 25 ml of 0.1% NaCl (w/v) was added, and then vortexed and centrifuged at 2500*g* for 20 min. The methanol/water layer was discarded and the $CHCl_3$ layer was taken to dryness *in vacuo*. Leaf material from [2-^{14}C]acetate labeling was extracted in a similar manner except three leaf discs with a combined area of 18.9 cm^2 were

Table III. *Amounts and Distribution of [^{14}C]Acetate in Various Glycerolipid Fractions from Soybean Leaves*

The leaves were either watered or stressed for 96 h of labeling. Values are the means of 3 replicates, 2 samples per replicate.

Treatment	GL	nCi·dm^2	% of Total
Control	PA + PI	0.73	4.5
Stress		0.87	10.7
Control	PC	0.86	5.3
Stress		0.79	9.7
Control	PE	1.81	11.2
Stress		0.73	9.0
Control	PG	2.89	17.8
Stress		1.55	19.0
Control	MGD	3.78	23.3
Stress		1.03	12.6
Control	DGD	5.32	32.8
Stress		2.03	24.9
Control	TG + DG	0.81	5.0
Stress		1.16	14.2
Control total		16.20	
Stress		8.16	
LSD 0.05		0.95	

lyophilized before extraction. Extraction of lyophilized plant tissues at low temperatures resulted in no differences in glycerolipid composition compared to homogenization in isoproponal hexane (3:2, v/v) in previous studies (15). Phospholipid and neutral lipid fractionation was achieved by TLC as described elsewhere (13). Fatty acid quantitation was performed by GLC equipped with a 2 m × 189 cm glass column packed with diethylene glycol succinate and a flame ionization detector. Heptadecanoic acid was used as an internal standard for quantitative analysis and to determine extraction and methylation efficiency.

[2-^{14}C]Acetate Labeling. Leaf tissue from the first trifoliate was labeled with [2-^{14}C]acetate 51.1 Ci mol-^{1} (New England Nuclear) by applying 100 μl (1 μCi) of acetate in ethanol to leaf tissue on the 1st d of witholding water. The [2-^{14}C]acetate was applied within 6.3 cm^2 circles marked on three leaves with a felt tip pen. Control tissue was treated with 100 μl ethanol. The glycerolipids were extracted and separated as described earlier. Radioactivity in the various fractions was measured by scraping the TLC bands into vials and adding 10 ml of a cocktail containing 3 L of toluene, 12 g PPO, and 0.5 g POPOP, then counting in a Packard 3330 scintillation counter. Quench corrections were made by the channels ratio method using $CHCl_3$ as a quenching agent.

RESULTS

Leaf water potentials decreased from −0.6 to −1.7 MPa after 4 d; then to −3.1 MPa after 8 d of withholding water. The amount of polar glycerolipids in stressed leaves were not altered until 8 d without water when a slight decrease was observed (Table I). (The amounts of DG and TG increased about 2-fold after 4 d of stress and TG increased 4-fold after 8 d of stress compared to the controls. The fatty acid composition of the TG and DG also changed after 4 d of stress. The amount of 18:3 increased 25% while palmitic (16:0) and oleic acids (18:1) decreased at least 50%. The changes in glycerolipids and fatty acid compositions were both reversed upon rewatering. After 8 d of stress, alteration of the fatty acid composition of the polar lipid fraction was also observed. The 18:3 content of this fraction decreased 22% while 16:0 and stearic (18:0) acid contents increased 53 and 27%, respectively.

Water stress also resulted in changes in the amounts of some phospholipids (Table II). The amounts of PA plus PI increased 4-fold, the amount of PC increased nearly 2-fold, and PE decreased slightly. There was little change in the amounts of PG, MGD, and DGD during this period of stress.

The first trifoliate of soybean plants incorporated externally applied [2-^{14}C]acetate into the fatty acids of all glycerolipids examined (Table III). After 4 d the control plants incorporated about 2-fold more radioactivity into glycerolipids than did the stressed plants. These results are in contrast to those obtained with water stressed cotton leaves which incorporated 25% more [^{14}C]acetate into glycerolipids than did controls (16). The distribution of label between glycerolipids was also altered by water stress. The percentage of label in the PA + PI and the PC fractions was increased 2-fold by water stress while the percentage of label in the PE and PG fractions from these treatments was similar. The percentage of label in the MGD fraction was decreased 46% and DGD labeling was decreased 24%. The percentage of label in the neutral lipid fraction (TG and DG) increased nearly 3-fold during water stress.

DISCUSSION

Increases in TG and 18:3 during water stress have been observed in corn leaf tissue (8). In chloroplasts isolated from water stressed barley and wheat leaves, decreased amounts of glycolipid and 18:3 were observed (6, 7). Soybean leaf tissue exposed to short-term water stress showed increases in TG and DG in response to decreased leaf water potential (Table I) and increased levels of 18:3 in the TG fraction. The observed increase in TG content was similar to the response to water stress of the drought susceptible soybean genotype Forrest (17). This alteration was reversed within 3 d after rewatering.

Alterations in amounts of the phospholipids PA + PI, PE, and PC were observed after 4 d of reduced leaf water potential. Leaf tissue labeled with [2-^{14}C]acetate reflected these changes to some degree as proportionately more label was incorporated into PA + PI and PC under stress and slightly less into PE. The increase in PC incorporation may have had implications in terms of glycinebetaine synthesis since choline is a precursor of glycinebetaine (11). No measurements of betaines were made in our study and existing evidence indicates betaine metabolism has not been studied in soybean (18).

The rate of [^{14}C]acetate incorporation into total fatty acids was slowed nearly 2-fold by drought stress (Table III) and synthesis of MGD and DGD were decreased 46 and 24%, respectively. Little change, however, was observed in the amounts of unlabeled MGD and DGD (Table II) during this time period. These results indicate that fatty acids in MGD and DGD may turn over slowly during drought stress. Since these galactolipids contained 73% of the fatty acids in the cell the changes observed in their biosynthesis relative to phospholipids were not apparent after short-term drought stress. This decrease in synthesis of chloroplast thylakoid lipids supports previous research which indicates a thinning of the cloroplast membranes at low water potential (9) which resulted in inhibition of electron transport and photophosphorylation (14). Maintenance of lipid synthesis at low water potentials may be a necessary key to maintaining chloroplast function.

The labeling data indicate that the elevated amounts of TG + DG in water stressed leaves are the result of increased *de novo* synthesis of these glycerolipids. While our data do not totally rule out breakdown of existing glycerolipids and reutilization of their fatty acids for TG and DG synthesis, the increases in

incorporation of ^{14}C into TG and DG and the relative stability of unlabeled galactolipids (Table II) make this possibility seem unlikely.

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