Plant Physiol. (1978) 61, 472–473

Short Communication

Relationship between Chloroplast Membrane Fatty Acid Composition and Photosynthetic Response to a Chilling Temperature in Four Alfalfa Cultivars[1]

Received for publication August 1, 1977 and in revised form October 23, 1977

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ABSTRACT

The photosynthetic responses of four alfalfa (*Medicago sativa* L.) cultivars to 10 and 22 C air temperatures were examined and the relationship between the photosynthetic response at 10 C and the fatty acid composition of the chloroplast membranes was determined. Chilling-resistant cultivars exhibited moderate reductions in photosynthesis at 10 C, compared to 22 C, and contained a significantly greater percentage of polyunsaturated fatty acids in the chloroplast membrane and a greater double bond index than the chilling-sensitive cultivars. The chilling-sensitive cultivars exhibited severe reductions in photosynthesis at 10 C, compared to 22 C. The reduction in photosynthesis at 10 C is shown to be negatively correlated ($r = -0.94$) with the double bond index of the chloroplast membranes of the cultivars observed.

The results support the hypothesis that reduced photosynthesis due to chilling temperatures is influenced by the unsaturated fatty acid composition of the chloroplast membrane which affect temperature-induced phase changes in chloroplast membrane lipids.

The inability of chilling-sensitive plants to maintain physiological activity at chilling temperatures has been correlated with a marked increase in the activation energy of respiratory enzymes (5, 10) and a marked increase in the activation energy of photoreduction of $NADP^+$ (7, 11). The observed increase in activation energy of these systems is related to temperature-induced phase changes in the lipids of the respective membranes (9–11). This phase change has been detected by a change in the mobility of spin labels and occurs at precisely the same temperature as the change in activation energy (8, 9). No change in activation energy was detected in respiratory enzymes (5, 10) or $NADP^+$ photoreduction (8, 11) of chilling-resistant plants, nor was a phase change in the lipids detected by spin labeling (8, 9). The ability of resistant plants to withstand chilling temperatures is correlated with a more flexible state of the membrane at these temperatures. This difference in the physical properties of membrane lipids from chilling-sensitive and chilling-resistant plants can also be correlated with the relative proportion of saturated and unsaturated fatty acids on the membrane lipids (2, 4, 6).

The present investigation was undertaken to examine the relationship between photosynthesis at a chilling temperature and the chloroplast membrane fatty acid composition of four alfalfa cultivars.

MATERIALS AND METHODS

Plant Material. Clonal propagules of four alfalfa (*Medicago sativa* L.) cultivars were established in 18-cm pots containing Charlton sandy loam with pH 6.8. The cultivars were selected on the basis of their general area of cultivation and chilling temperature response. 'Kane' alfalfa is primarily cultivated in Canda, 'Vernal' in northern U.S., 'Moapa' and 'Mesa-Sirsa' in southwestern U.S. Kane and Vernal are resistant to chilling temperatures and Moapa and Mesa-Sirsa are chilling-sensitive. The plants were arranged in a completely randomized block design with four replications in a greenhouse at 25/18 C (day/night temperatures). Each plant was watered daily and received 25 ml of 1% aqueous 20-20-20 fertilizer solution every 14 days. At the time of each analysis, the plants had attained 30 days regrowth.

Chloroplast Extraction. Chloroplasts were isolated from depetiolated leaves of each plant, three replications, in extraction buffer (0.4 M sucrose, 0.05 M Tricine, 0.01 M NaCl, pH 7.8) (1). The leaves were homogenized in a VirTis "23" homogenizer for 10 sec at maximum speed and 30 sec at 0.5-speed in 40 ml of extraction buffer. The suspension was filtered through four layers of cheesecloth and the filtrate centrifuged for 90 sec at 500*g*. The pellet was discarded and the supernatant recentrifuged for 7 min at 1,000*g*. The resulting pellet was resuspended in 20 ml of extraction buffer, centrifuged for 90 sec at 500*g*, and the pellet discarded. The supernatant was recentrifuged for 10 min at 2,000*g* and the resulting pellet dissolved in 25 ml of methanol and 5 ml of methylene chloride. This solution was directly analyzed for fatty acid composition. The chloroplast pellet was found to contain 90% intact chloroplasts, as judged by light microscopy.

Chloroplast Fatty Acid Assay. Methyl esters were prepared by direct micromethanolysis using boron trichloride gas (3). Qualitative and quantitative analyses were performed according to established gas-liquid chromatographic procedures (3, 7). The column was calibrated with Applied Science (State College, Pa.) fatty acid standard K-102 and agreed with the composition of the chloroplast samples with a relative error less than 10% for major components (>10% of the total mixture) and less than 10% for minor components (<10% of the total mixture). Standard fatty acid methyl esters showed a linear response over the range of sample sizes analyzed. Each peak was identified and labeled according to the ratio of the number of carbon atoms to the number of double bonds in the molecule. The double bond index, the summation of the relative percent of each acid multiplied by the number of double bonds it contains per molecule and divided

[1] Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 879.
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by 100, was used to indicate the degree of unsaturation for the membrane (6).

Photosynthetic CO_2 Exchange Determinations. Photosynthetic CO_2 exchange was measured on individual plants in an open system utilizing a Plexiglas chamber (35 × 35 × 20 cm), equipped with a heat exchanger, and a Beckman model 865 IR gas analyzer. Air was supplied to the chamber at a flow rate of 4 liters/min and contained approximately 300 μl/l CO_2. Illumination was provided by eight 150-w floodlamps which supplied a photosynthetic photon flux density of 850 $\mu E/m^2 \cdot$ sec (400–700 nm) at plant height. The plants were placed in the chamber and photosynthetic CO_2 exchange measurements were taken at 22 and 10 C. During all measurements, the roots were maintained at 22 C. Each measurement required 45 min for CO_2 and temperature equilibration. Photosynthetic CO_2 exchange measurements at each temperature were replicated four times/cultivar.

RESULTS AND DISCUSSION

Table I shows the response of the four alfalfa cultivars to 10 C and 22 C air temperatures. The reduction in photosynthetic CO_2 exchange at 10 C by Kane and Vernal was less than the reduction exhibited by Moapa and Mesa-Sirsa. Moapa and Mesa-Sirsa are adapted to hot environments and are chilling-sensitive alfalfa cultivars. The chilling-resistant cultivars, Kane and Vernal, exhibited only moderate reductions in photosynthetic CO_2 exchange at 10 C, the former being less affected.

The fatty acid composition of chloroplast membranes of the four alfalfa cultivars is shown in Table II. A significant variation in fatty acid composition is noted among cultivars. Kane and Vernal contained significantly less 16:1 and significantly more 18:3 than Moapa and Mesa-Sirsa. A greater percentage of polyunsaturated fatty acids were observed in the chloroplast membranes of Kane and Vernal. The double bond indices were also greater in Kane and Vernal.

The relationship between the double bond index of the chloroplast membranes and the per cent reduction in photosynthetic CO_2 exchange at 10 C is shown in Figure 1. The double bond index and chilling-induced reductions in photosynthetic CO_2 exchange were negatively correlated ($r = -0.94$) for the four alfalfa cultivars observed. Mitochondrial membrane fatty acid data also indicated that mitochondria isolated from chilling-resistant plant tissue have a greater degree of unsaturated fatty acids, as indicated by the double bond index, then mitochondria from chilling-sensitive tissue (6).

The results presented in this paper show that the physical nature of the chloroplast membranes of chilling-sensitive and chilling-resistant cultivars of alfalfa did differ. The greatest degree of unsaturation of the membrane fatty acids was found in chloroplasts from chilling-resistant cultivars, and the lowest values were found in chilling-sensitive cultivars. The degree of unsaturated fatty acids in the chloroplast membranes of alfalfa cultivars, represented by the double bond index, was negatively correlated with the reduction in photosynthetic CO_2 exchange at 10 C, compared to 22 C. These results agree with the hypothesis that the degree of unsaturation of membrane fatty acids influences the sensitivity of the membrane to chilling temperatures (2, 4, 6).

Table I. The effect of air temperature on the photosynthetic CO_2 exchange of four alfalfa cultivars

Air Temperature	22 C	10 C	Reduction
	mg $CO_2/dm^2/hr$		%
Cultivar			
Kane	28.83 b[1]	21.10 c	26.8
Vernal	36.55 a	21.28 c	41.8
Moapa	22.82 c	6.32 d	72.3
Mesa-Sirsa	30.95 b	8.59 d	72.3

[1] Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table II. The relative fatty acid composition of the chloroplast membranes isolated from four alfalfa cultivars

Fatty acid	Relative percent of total fatty acid content			
	Kane	Vernal	Moapa	Mesa-Sirsa
16:0	9.1 a[1]	8.8 a	3.2 a	6.4 a
16:1	6.1 a	24.1 b	57.0 c	38.2 d
18:0	3.1 a	3.5 a	2.2 a	3.2 a
18:1	0.8 a	1.4 a	0.0 a	1.0 a
18:2	8.2 a	3.9 b	3.7 b	4.5 b
18:3	72.6 a	57.8 b	33.1 c	46.6 d
Saturated %	12.2	12.3	5.4	9.6
Monounsaturated %	6.9	25.5	57.0	39.2
Polyunsaturated %	80.8	61.7	36.8	51.1
Double Bond Index	2.41	2.07	1.64	1.88

[1] Means within a row followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

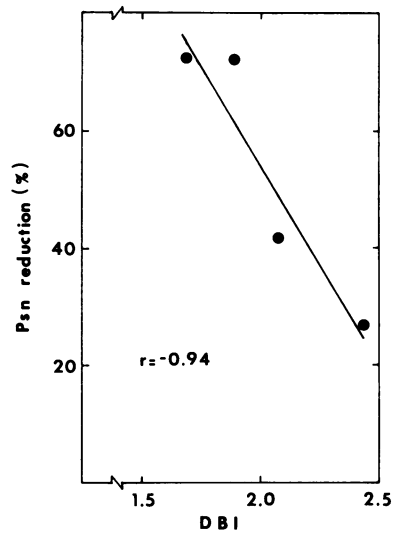

FIG. 1. Relationship between the double bond index (DBI) of chloroplast membrane fatty acids and the per cent reduction in photosynthesis at 10 C compared to 22 C in four alfalfa cultivars. Each point represents the mean of four measurements.

These data also indicate that cultivars within a species vary as to the fatty acid composition of chloroplast membranes, and this may be related to the chilling response of the cultivar. Breeding for a higher degree of unsaturated fatty acids (higher double bond index) within a species may increase the resistance of a cultivar to chilling injury.

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