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RAPID DEGRADATION OF POLAR LIPIDS IN FROST DAMAGED WINTER WHEAT CROWN AND ROOT TISSUE*

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Key Word Index—*Triticum aestivum*; Gramineae; winter wheat; frost; triglycerides; diglycerides; polar lipids; polyunsaturated fatty acids; degradation.

Abstract—The effect of a lethal frost on the lipid composition of crown and root tissue of winter wheat after thawing was investigated. The data show rapid degradation of polar lipids with an increase first in diglycerides and later in unesterified fatty acids, while active synthesis of triglycerides was maintained during the first 6 hr after thawing. Polyunsaturated species of polar lipids were preferentially degraded. Extensive losses of total linoleic and linolenic acids suggest lipoxygenase action. The observed lipid degradation after frost is probably the result of decompartmentation following damage to membranes.

INTRODUCTION

When fatty acid composition of the lipids of root and crown tissue of winter wheat was determined during frost hardening, no difference could be demonstrated between cultivars differing widely in frost tolerance [1, 2]. Could differences in frost hardiness be partly explained in terms of rate of degradation of membrane lipids after frost damage and potential of the tissue for repair? As a first step in this investigation, degradation of lipids after a killing frost was described.

RESULTS

Within 6 hr of thawing after a killing frost, one-third of the lipid phosphorus was lost from winter wheat root and crown tissue; two-thirds were lost after 24 hr (Table 1). Extensive degradation of total fatty acids also occurred during this time (Table 1), particularly of linoleic and linolenic acids (Fig. 1). On a percentage basis, linolenic acid content decreased from 26 to 17% and palmitate increased from 22% to 28% during 24 hr.

Polar lipid breakdown accounted for these losses (Fig. 2). Their fatty acid content was lowered from 520 to 170 μg/g fr. wt, and their percentage of total fatty acids from 79 to 52%. The molar ratio of polar lipid fatty acid to lipid phosphorus, assuming an average MW of 275 for the fatty acids, was 2.08 before freezing, 1.73 before thawing, 1.65 after 6 hr of thawing and 1.88 after 24 hr. Diglycerides and triglycerides increased early in the thawing period and unesterified fatty acids later. Diglycerides went up from 24 μg fatty acid/g fr. wt before freezing to 48 μg/g fr. wt after 6 hr of thawing ($P < 0.01$) or from 4 to 11% of total fatty acids ($P < 0.01$). Triglyceride content rose during the same period from 88 to 114 μg/g fr. wt ($P < 0.05$) or from 14 to 26% of total fatty acids ($P < 0.05$). Unesterified fatty acids showed a decrease from 27 to 16 μg/g fr. wt early in the thawing period, but increased to 41 μg/g fr. wt after 24 hr ($P < 0.01$).

Table 1. Lipid phosphorus and total fatty acid content of crown and root of winter wheat before a lethal frost at −10° (control) and after 0, 6 or 24 hr thawing

	Lipid phosphorus*		Total fatty acids*	
Sample	Fr. wt (μg/g)	% of control	Fr. wt (μg/g)	% of control
Control	28.7 ± 1.5[a]	100.0 ± 5.2	795.1 ± 102.9[a]	100.0 ± 13.0
0 hr	24.8 ± 1.6[b]	86.5 ± 5.6	714.4 ± 151.7[a]	89.9 ± 19.1
6 hr	18.9 ± 3.1[c]	65.8 ± 10.8	583.5 ± 157.0[ab]	73.4 ± 19.8
24 hr	10.4 ± 0.6[d]	36.3 ± 2.3	479.9 ± 57.0[b]	59.1 ± 7.2

*Average of four samples ± s.d. Different letters after numbers in the same column mean a difference significant at the 5% level.

Analysis of the fatty acids of these groups of lipids showed appreciable loss of polyunsaturated fatty acids in the polar lipids, with a corresponding enrichment in

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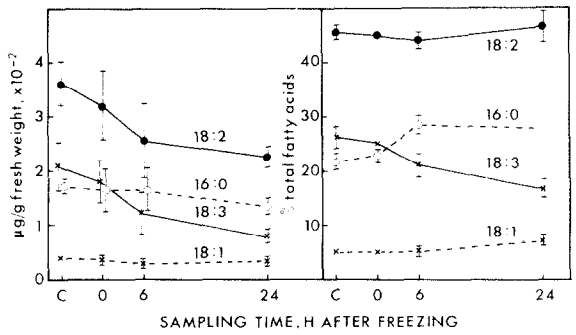

Fig. 1. Total fatty acid content of root and crown of winter wheat before a lethal frost (control, C) and after 0, 6 or 24 hr thawing. Chain length: No. of double bonds: palmitate, 16:0; oleate, 18:1; linoleate, 18:2; linolenate, 18:3. Average of four samples; vertical lines show s.d., when large enough.

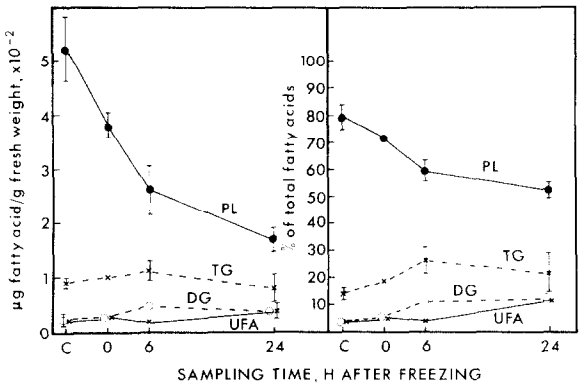

Fig. 2. Fatty acid content of groups of lipids of root and crown of winter wheat before a lethal frost (control, C) and after 0, 6 or 24 hr thawing. Average of four samples ±s.d. PL, polar lipids; DG, diglycerides; UFA, unesterified fatty acids; TG, triglycerides.

palmitate (Fig. 3). Diglycerides showed little change in their fatty acid composition. Triglyceride linolenate content rose significantly from 6.0 µg/g fr. wt before freezing to 12.3 µg/g fr. wt immediately after freezing ($P < 0.01$) and from 12.3 to 18.3 µg/g fr. wt after 6 hr of thawing ($P < 0.05$) [6.7, 12.5 ($P < 0.01$) and 16.0% of the triglyceride fatty acids, respectively]. Although the triglycerides did not become enriched in linoleate, this fatty acid increased in the fraction from 47 to 62 µg/g fr. wt in 6 hr ($P < 0.01$), reflecting the rise in triglyceride content. Unesterified fatty acids were very rich in palmitate and this fatty acid increased further upon thawing. This fraction did not contain linolenic acid and only a little linoleic acid.

DISCUSSION

The data presented above indicate a rapid degradation of polar lipids after stress and an increase, first in diglycerides, then in unesterified fatty acids, while active synthesis of triglycerides was maintained during the first hours after thawing.

Lipid phosphorus and polar fatty acids decreased from 28.7 to 18.9 µg/g fr. wt and from 519.3 to 261.4 µg/g fr. wt,

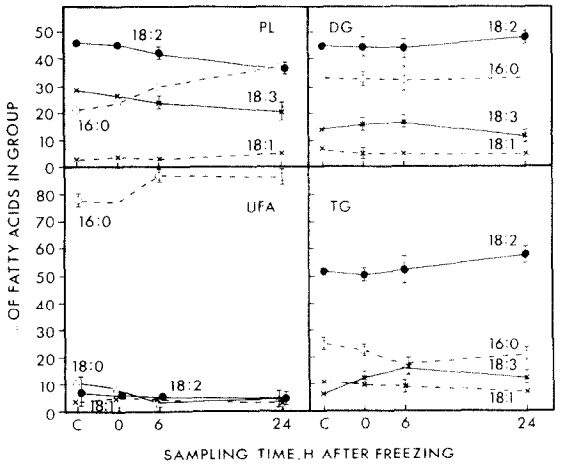

Fig. 3. Fatty acid composition of groups of lipids of root and crown of winter wheat before a lethal frost (control, C) and after 0, 6 or 24 hr thawing. Average of four samples ±s.d. For explanation of symbols, see Fig. 1 and 2. Stearate, 18:0.

respectively, during the first 6 hr after stress. The significant rise in diglyceride content after thawing suggests that lipid degradation was mediated sequentially by two hydrolases, phospholipase D and phosphatidic acid phosphatase, as described by Herman and Chrispeels [3]. Freezing was shown to trigger phospholipase D activity in soybean cotyledons [4] and in cortical tissue of poplar [5] and black locust [6]. A large proportion of the diglycerides produced, especially those containing linolenate, was incorporated into triglycerides. Actual synthesis of triglycerides during this period was indicated by the significant increase of linolenate content in the group during freezing ($P < 0.01$) and during the first 6 hr after thawing ($P < 0.05$), when expressed as µg/g fr. wt.

The rapid lowering of total linoleate and linolenate content and the absence of linolenate, and the very low level of linoleate in the unesterified fatty acid fraction, suggest rapid lipoxygenase action on fatty acids liberated by acid hydrolases. The decrease in total linolenate was accounted for by loss of linolenic acid from the polar lipid fraction. The hydrolases which degraded the polar lipids to diglycerides attacked preferentially the less saturated species, including the galactolipids. The content of galactolipids, which should push the molar ratio of fatty acids to phosphorus to higher values than the theoretical 2.00 for phosphatides, was apparently small and almost offset by the presence of lysophosphatides. The lowering of the ratio early after freezing probably reflects degradation of galactolipids and some deacylation to lysophosphatides.

The data from these experiments are thus interpreted as showing rapid formation of mainly polyunsaturated diglycerides from polar lipids. A part of these diglycerides was incorporated into triglycerides, while the rest were further degraded to unesterified fatty acids, among which the polyunsaturated ones were rapidly degraded by lipoxygenase action.

Our data show much analogy with the results of Rodionov and Zakharova [7] obtained with homogenates of potato leaves and those of Theologis and Laties [8] obtained with potato tuber slices. These authors measured extensive loss in lipid phosphorus and poly-

unsaturated fatty acids in the absence of a low temperature stress. The observed lipid degradation after frost is, therefore, probably the result of decompartmentation following damage to membranes [9].

EXPERIMENTAL

Triticum aestivum L. cv Kharkov 22 MC was grown in 10-cm pots containing a 1:1 (v/v) sand–vermiculite mixture in a growth cabinet at 20°/15° day/night temp with a 16 hr photoperiod, r.h. 60%, irradiance of 160 μE/m^2·sec. The plants were watered with Hoagland No. 1 soln [10].

12-day-old plants were freed from potting mixture. After removal of the shoots, the roots and crowns were weighed and placed in plastic bags, five plants per bag, with a moist piece of paper. They were equilibrated at +0.5° in a programmed freezer, cooled to −4° at 2°/hr, kept at this temp. for 9 hr to allow equilibration, and cooled further to −10°. After 2 hr at −10°, the bags were removed from the freezer, slightly opened for aeration and allowed to thaw at room temp. Samples were collected before freezing and after 0, 6 and 24 hr thawing. They were boiled for 3 min in H_2O and covered with 5 ml hexane–isopropanol (3:2) [11].

Tissues were homogenized (Polytron) and the homogenate was filtered over glass wool. The residue was rinsed twice with 3 ml of the extraction solvent. The combined extracts were washed with 4.4 ml Na_2SO_4 (6.5%, w/v). The upper phase was evaporated to dryness in N_2 and redissolved in 0.5 ml $CHCl_3$. Lipid phosphorus was determined on two 40 μl aliquots according to the micromethod of Bartlett [12] as modified by Kates [13]. Total fatty acids were determined on 100 μl aliquots after transmethylation in 14% (w/v) BF_3 in MeOH (Applied Science) and GC of the fatty acid methyl esters [14]. Lipids were separated into polar lipids, diglycerides, unesterified fatty acids and triglycerides by TLC on Si gel G in hexane–Et_2O–HOAc (80:20:1) with suitable reference compounds (Applied Science) and detected by brief exposure to I_2 vapour. After separation, unesterified fatty acids were methylated in the BF_3 reagent and the other lipids were transmethylated in 0.5 M NaOMe in MeOH [15], on the gel.

Two expts were carried out. The first one, with quadruplicate sampling, is described. The results of the second showed the same trends. The variances of the variables of interest (lipid phosphorus, polar lipids, diglycerides, unesterified fatty acids and triglycerides) increased with the mean. The data were thus transformed to the logarithmic scale before analysis. Duncan's multiple range tests were applied to differences between treatment means (control, 0, 6 and 24 hr of thawing).

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