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ADAPTATION OF MEMBRANE FLUIDITY OF RYE AND WHEAT SEEDLINGS ACCORDING TO TEMPERATURE

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Key Word Index—*Secale cereale; Triticum aestivum*; Gramineae; membrane fluidity; cold resistance; chloroplasts; fatty acids; cryoprotective substances.

Abstract—Order parameters of chloroplast membrane lipids of rye and wheat seedlings differing in cold hardiness were compared before and after hardening. Seedlings grown at 25° exhibited similar membrane microviscosities. When hardened, the cultivars most resistant to freezing temperatures possessed the most fluid membranes, while those sensitive to cold were unable to alter them. Changes in linolenic acid levels alone cannot be responsible for the observed phenomena.

INTRODUCTION

It is well accepted that membranes are in a liquid–crystalline state at body temperature [1] and there are good reasons to believe that the fluidity of these structures is a function of temperature [2, 3]. The ability to adapt the physico-chemical state of membranes to the temperature can be regarded as important for survival at low temperatures by both poikilothermic animals and plants. Some wheats can tolerate low temperatures while others cannot; the former acquire their resistance during hardening [4]. It is a reasonable expectation that while exposed to cold these plants gradually increase the fluidity of their membranes, and their survival ability can be related to the fluidity that these structures attained. This paper compares the fluidity of several warm adapted and hardened rye and wheat seedlings.

RESULTS AND DISCUSSION

In Table 1 the order parameters, as measured using the spin label I(12, 3) of the chloroplast lipids of 5 grain seedlings of different hardinesses, are compared with their survival abilities at sub-zero temperatures. The order parameters of their structural lipids were within a narrow range when grown at 25°. Based on this observation and on the above speculation, one would also expect that the membranes of the hardened seedlings would exhibit lower but similar values. Despite the identical treatment, however, the membranes of the plants differed with respect to their fluidities at the end of the hardening process. Those most resistant to freezing temperatures possessed the most fluid membranes and the acquired fluidity was directly related to their abilities to survive in the cold. Short mexican, of which only 4% survived under the conditions of the test, did not change its membrane fluidity during hardening and in Penjamo 62, with 0% survival, the membranes became even more rigid. If we assume that the optimal membrane fluidity was attained by *Secale cereale* and Miranovskaja 808, it will be evident that the extent of low temperature toleration is connected with the success of the plants to adjust the membrane fluidity to the prevailing temperature. From Table 1 it can be seen that 50% survival can be achieved with membranes exhibiting an order parameter of *ca* 0.73.

Table 1. Order parameters (S_{33}) of chloroplast lipids and frost tolerance of rye and wheat seedlings

Seedling	S_{33} UH	S_{33} H	Frost tolerance H (survival at −18°)
Secale cereale	0.761	0.636	100
Miranovskaja 808	0.778	0.625	88.5
Bezostaja 1	0.783	0.716	67.3
Short mexican	0.776	0.765	4.0
Penjamo 62	0.793	0.874	0

UH: Unhardened; H: hardened.

The most common way to adjust membrane fluidity to decreasing temperatures is to alter the unsaturation of the structural lipids. An increase in the level of linolenic acid during hardening was reported in several cases [5, 6], although the importance of this fatty acid has recently been doubted [6, 7]. We observed a reduction in the level of linolenic acid in chloroplasts of sensitive cultivars (Short mexican and Penjamo 62) during hardening, but rye and resistant cultivers did not show increased amounts of this acid while exposed to cold. It is evident that *S. cereale*, Miranovskaja 808 and Bezostaja 1 increased fluidity without accumulating more linolenic acid in their membranes which can be regarded as strong evidence of non-involvement of polyunsaturated fatty acids in adaptation processes (Table 2). The amount of linolenic acid in Bezostaja 1, with a cold tolerance of 67‰, was 10% higher than that in *S. cereale*, of which 100% survived under identical conditions. If the linolenic acid level or the unsaturation index alone determined the membrane fluidity and the frost resistance, Bezostaja 1 was in a more advantageous position than either *S. cereale* or Miranovskaja 808.

Table 2. Changes in fatty acid composition of chloroplast lipids in the course of hardening

Seedling	Fatty acids (wt %) 16:0		16:1		18:0		18:1		18:2		18:3	
	UH	H	UH	H	UH	H	UH	H	UH	H	UH	H
Secale cereale	17.8	17.0	3.5	4.4	3.5	1.9	3.1	4.0	8.1	9.9	63.9	62.8
Miranovskaja 808	16.6	15.3	4.1	4.7	2.4	1.3	4.3	4.4	7.0	12.2	65.4	62.1
Bezostaja 1	13.9	10.8	3.1	2.9	1.5	1.2	3.0	3.4	6.3	9.6	72.1	72.2
Short mexican	15.7	27.1	4.8	5.1	1.8	3,3	3.5	7.2	6.4	11.5	67.7	45.7
Penjamo 62	17.4	18.0	4.6	7.4	2.1	2.7	5.4	7.9	7.2	10.4	63.1	53.7

UH: Unhardened; H: hardened. The values are the mean of 3 determinations.

Moreover, the average unsaturation of chloroplasts was significantly higher in Penjamo 62 than in Short mexican, despite the fact that the former produced more rigid membranes.

The observed degradation of membranes in chill and freeze injured cells [8, 9] is very likely due to the liberation of phospholipase D at the temperature of phase transition and the consequent decomposition of phospholipids. The extent of the accumulation of phosphatidic acid can be regarded as an indicator of membrane damage. In Table 3, the content of phosphatidyl choline (PC) and phosphatidic acid (PA) as well as the ratio of PA to PC is compared in hardened seedlings. This ratio increases from 0.176 in *S. cereale* to 0.769 in Penjamo 62 and in general there is a direct relationship between the capability of preserving membrane integrity and survival at low temperatures. The fact that the PA/PC ratio was also inversely related to the order parameters emphasizes the basic importance of the transitional state of membrane lipids in avoiding freezing injury in the cold. Plants which cannot provide optimum fluidity to their membranes will be handicapped when the temperature decreases.

Table 3. Phosphatidyl choline and phosphatidic acid content of leaves of hardened rye and wheat seedlings

Seedling	PC	PA	PA/PC
	10^{-7} mol/g leaf		
Secale cereale	11.9	2.1	0.176
Miranovskaja 808	14.5	6.2	0.427
Bezostaja 1	15.0	7.7	0.513
Short mexican	10.3	8.3	0.806
Penjamo 62	11.7	9.0	0.769

PC = Phosphatidyl choline; PA = phosphatidic acid.

At present we cannot explain how cold resistant plants assure the optimum fluidity of their membranes in the cold. We can only postulate that they produce certain cryoprotective substance(s) during hardening. These substances should be of lipidic nature to insert into the bilayers and prevent the ordering of hydrocarbon chains at the critical temperature. Differences in the intensity of production of these compounds and hence the protection of membranes should be different in wheats of different hardiness, and can determine the ability to withstand low temperatures.

EXPERIMENTAL

Seedlings of *S. cereale*, wheat cv Miranovskaja 808, Bezostaja 1, Short mexican and Penjamo 62 were used. The sowing took place in late October in the Institute's experimental field, the investigations being carried out in mid-January. By this time all the plants could be regarded as hardened. When grown in the laboratory, they were germinated on wet filter papers at 25° and illuminated by a 100 W bulb; 6-day-old seedlings were used. Details of the cold test will be described in a forthcoming paper. Chloroplast isolation was done according to the method of ref. [10].

Separation and analysis of lipids. To inactivate phospholipase D, the leaves were boiled with *iso*PrOH for 15 min and then homogenized in $CHCl_3$–MeOH (2:1). Chloroplast lipids were treated only with the latter extraction system. The determinations of the phospholipid composition of the leaves, of the fatty acid composition of chloroplast lipids, and of the order parameters were carried out on the washed $CHCl_3$ phase. The phospholipids were separated by 2D-TLC [11] and the lipid P determination was determined using the method of ref. [12]. Fatty acid Me esters were obtained by transesterification in MeOH containing 5% HCl at 80° in sealed ampoules. The GLC separation was performed on SP-2340 (Supelco).

ESR studies. The extracted lipids were labelled with the fatty acid spin label I(12, 3) (2-(3-carboxypropyl-4,4-diMe-2-2-tridecyl-3-oxazolidinyl-oxyl, SYVA), as described in ref. [13], in a $CHCl_3$ soln. A typical lipid to label ratio was 100:1. After evapn of the solvent, the lipid film was incubated with a buffer soln (pH 7.4) in closed vials. The lipid dispersions obtained were measured in a randomly oriented bulk state [14]. The measurements were performed using a JEOL JES-PE-1x spectrometer. For calculation of the order parameter (S_{33}) a polarity correction was made [15].

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