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Changes in fatty acids of leaf polar lipids during chilling and post-chilling rewarming of *Zea mays* genotypes differing in response to chilling

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Abstract

Changes in fatty acids of leaf polar lipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) in maize seedlings of chiling-sensitive (CS) CM 7 and Co 151 lines and chilling-tolerant (CT) S 215 and EP 1 lines upon chilling for either 4 or 6 days in the dark and after rewarming for 4 days at original growth conditions were studied. The content of free fatty acids (FFA) in control leaves as well as alterations in the proportion of major fatty acids, unsaturation ratio (UR), double bond index (DBI) and changes in the proportion of heigh-temperature melting of both phosphatidylglycerol (htm-PG) and sulfoquinovosylglycerol (htm-SQDG) after chilling and rewarming of seedlings were estimated.

FFA content in intact leaves was 2-3-fold higher in the chilling susceptible CM 7 line than in the other three inbreeds studied. After chilling for 6 days the level of FFA increased only in CM 7 and S 215 lines by about 30 %. Upon rewarming seedlings chilled for 6 days the level of FFA increased about twofold in CS Co 151 line and CT EP 1 line and decreased in CS CM 7 line. Limited accumulation of FFAs during chilling and post-chilling rewarming of maize seedlings, did not correspond to the extent of polar lipid breakdown (Kaniuga *et al.* 1999b) probably due to the contribution of active oxidative systems to the peroxidation of fatty acids under these conditions.

During rewarming seedlings chilled for 6 days major changes were observed in decrease of 18:3 and an increase of 16:0 in all four polar lipids studied with more pronounced changes in CS than CT lines. Similarly, in CS inbreeds a decrease in UR of fatty acids in MGDG, DGDG and SQDG after post-chilling rewarming was greater than in CT lines. Proportion of htm-fraction in both PG and SQDG increased after post-chilling rewarming in all four inbreeds, however to a lesser extent in CT than CS lines. A similar pattern of changes in DBI in CS and CT maize seedlings was observed in glycolipid and combine lipid classes.

More extensive degradation of polar lipids in CS than CT maize inbreeds following galactolipase action in chloroplasts (Kaniuga *et al.* 1998) provides FFAs for initiation of peroxidation by LOX which is manifested by decrease of UR and DBI. This sequence of reactions during chilling and post-chilling rewarming appears to be a main route of fatty acids peroxidation responsible for secondary events involved in chilling injury. In addition, the extent of these changes differentiates CS and CT inbreeds. Contribution of esterified fatty acids in thylakoid lipids to direct peroxidation, may be of minor importance.

List of abbreviations: Chl = chlorophyll; CS, CR and CT = chilling sensitive, - resistant and - tolerant; DGDG = digalactosyldiacylglycerol; FFA = free fatty acids; htm-PG = high temperature melting fraction of PG; LAH = lipid acyl hydrolase (galactolipase) EC 3.1.1.26; LOX = lipoxygenase EC 1.13.11.12; MGDG = monogalactosyldiacylglycerol; PG = phosphatidylglycerol; SOD = superoxide dismutase EC 1.15.1.1; SQDG = sulfoquinovosyldiacylglycerol; 16:0 = length of carbon chain:

number of double bond; 16:1 *t* = hexadeca-*trans*-3--enoic acid; $O_2^{\cdot-}$ = superoxide anion radical

Introduction

Deleterious effect of low temperature on chilling susceptible plants accompanied by disruption of several metabolic processes is manifested by visible chilling injury of tissue. For more precise characterization of these changes, Raison and Lyons (1986) proposed distinction of two consecutive stages of chilling injury. The first, termed "primary event" is physical in nature and temperature dependent and related to "sensitivity" to chilling. The second one is time dependent and includes a number of degradative processes, among which alternations in the ordering of membrane lipids are the most essential. These degradative precesses are initiated or accelerated by chilling were termed "secondary" events. Extension of these changes is usually manifested by magnitude of visible symptoms. This stage may be referred to as "response" to a chilling stress (Raison and Orr, 1990).

The effect of chilling is correlated with both the proportion of htm-PG in leaf polar lipids (Raison and Wright, 1983; Murata and Yamaya, 1984; Orr and Raison, 1987) and critical temperature for photosynthesis (Hodgson *et al.*, 1987; Raison and Brown, 1989). Our studies have indicated, however, that despite equal level of htm-PG in CS and CT seedlings of maize (Kaniuga *et al.*, 1999a) a different "response" to chilling stress was observed upon assessement of visual injury symptoms after post-chilling rewarming correlated with both enhanced degradation of leaf polar lipids (Kaniuga *et al.*, 1999b) and more active LAH (galactolipase) in chloroplasts of CS than CT inbreeds (Kaniuga *et al.* 1998). These results suggested that hydrolytic breakdown of polar lipids followed by release of fatty acids facilitate their effective peroxidation which is considered as an important factor responsible for chilling in plant tissues (*cf.* Parkin *et al.*, 1989; Parkin and Kuo, 1989; Hariyadi and Parkin, 1993).

The aim of the present work was to compare changes in fatty acid content and composition during chilling and post-chilling rewarming of CS and CT seedlings of maize and to relate them to the extent of polar lipid degradation under these conditions (Kaniuga *et al.* 1999b).

Material and Methods

Plant materials

Selection of CS maize inbred lines (CM 7 and Co 151) and CT lines (S 215 and EP 1) seed germination as well as conditions of seedlings growth for 14 days are given in previous paper (Kaniuga *et al.*, 1999b). Both chilling of seedlings at 5 °C in dark for either 4 or 6 days followed by rewarming for 4 days at original growth conditions as well as visual assessment of post-chilling injury are also described (Kaniuga *et al.*, 1999b).

Analysis of MGDG, DGDG, SQDG PG and FFA

Lipids were extracted from lyophylized secondary leaves of maize seedlings. The dried material was first boiled for 10 min in isopropanol/0.73 % NaCl in water. Extraction of lipids was performed according to Bligh and Dyer (1959) using isopropanol and aqueous NaCl solution. Individual lipids were separated by two-dimensional TLC (Douce *et al.*, 1990) as previously described (Kaniuga *et al.*, 1999b). FFA were purified from lipid extracts by TLC on precoated silica gel G plates (Merck 5721) using hexane : diethyl ether : acetic acid (70:30:1, by vol.). After detection with 0.02 % (w/v) of 2,7-dichlorofluorescein in methanol, the spots of MGDG, DGDG, SQDG and PG or the bands of FFA were scraped off.

Glycerolipids were transmethylated and FFA methylated on thin-layer adsorbent (Christie, 1989) in the presence of internal standard (pentadecanoic acid) and resultant fatty acid methyl esters were analysed by gas-liquid chromatography as previously described (Kaniuga *et al.*, 1999b). Methyl esters of fatty acids were identified with authentic reference standards (provided by Sigma) and quantified by comparison of peak areas to those of internal standards. Determination of individual lipid class was performed in 3 replicates.

Results

Free fatty acid level

In the intact leaves of three inbred lines the content of FFA was between 0.16 and 0.30 while in more chilling susceptible CM 7 line it amounted to 0.62 $\mu mol \cdot mg^{-1}$ Chl (Table 1). Upon dark chilling of maize seedlings for 6 days changes in the FFA level were very small in three inbreeds, except that in the most chilling susceptible CM 7 line (Table 1).

Table 1. Changes in the FFA content in leaves during chilling of maize seedlings for 4 and 6 days in the dark. Values express the means of FFA content in $\mu mol \cdot mg^{-1}$ Chl ± SE for n=3.

Breeding line		Control	Days of chilling	
			4	6
CS	CM 7	0.62 ± 0.06	0.62 ± 0.07	0.94 ± 0.07
	Co 151	0.28 ± 0.09	0.25 ± 0.03	0.22 ± 0.30
CT	S 215	0.16 ± 0.02	0.14 ± 0.01	0.24 ± 0.05
	EP 1	0.30 ± 0.08	0.25 ± 0.05	0.20 ± 0.02

Changes in the FFA level upon rewarming of maize seedlings following their chilling for 4 or 6 days are shown in Table 2. In seedlings chilled for 4 days a small increase of FFA level was noticed in all inbreeds after rewarming for 4 days, even in the most chilling susceptible CM 7 line containing about threefold higher level of FFA in control leaves. When chilling of seedlings was extended up to 6 days the level of FFA increased in CM 7 and S 215 lines. In contrast, FFA level decreased in EP 1 line and did not alter in Co 151 line. More significant changes in FFA level were found upon rewarming of maize inbreeds subsequently to chilling for 6 days (Table 2). An increase of FFA level was observed in Co 151 and EP 1 lines, while in CM 7 and S 215 lines a decrease and no changes were noticed, respectively. The changes in FFA level in chilled and rewarmed maize seedlings may suggest no correlation between FFA content and the extent of total polar lipid degradation found previously (Kaniuga *et al.*, 1999b) presumably due to various activity of oxidative systems in individual inbreeds involved in FFA catabolism.

Changes in the proportion of major fatty acids

As shown in Table 3 following both chilling and rewarming the highest increase in the proportion of 16:0 was found in PG and SQDG accompanied by almost similar decrease of 18:3 in these two polar lipids. In addition, changes in the proportion of 16:0 and 18:3 were greater in CS than in CT inbreeds. In PG a decrease (-Δ) of 16:1 *tr* + 18:3 was approximately compensated by an increase (+Δ) of 16:0 + 18:2 while in MGDG, DGDG and SQDG a decrease (-Δ) of the proportion of 18:2 + 18:3 was roughly similar to that of 16:0 with the exception of some increase of 18:2 in MGDG and DGDG in line CM 7. It is interesting that despite similar level and composition of polar lipids in CS and CT inbreeds of maize (Kaniuga *et al.*, 1999a) stress conditions differentially affected membrane lipid composition. These differences might be related to various galactolipase activity in CS than CT lines and to greater abundance of galactolipids than other polar lipids.

Changes in unsaturation ratio and htm-fraction in PG

Data in Table 4 show decrease of UR in MGDG, DGDG and SQDG. Decrease of UR in MGDG during chilling was small (about 10-20 %) in comparison to the values measured in non-chilled samples.

Table 2. Changes in the FFA content in leaves during post-chilling rewarming for 4 days of maize seedlings subsequently to chilling for 4 and 6 days. Values express the means of FFA content in $\mu mol \cdot g^{-1}$ DW ± SE for n=3.

Treatment		Inbreed line			
		CS		CT	
Chilling	Days	CM 7	Co 151	S 215	EP 1
Chilling	4	11.15 ± 2.36	2.94 ± 0.35	2.23 ± 0.18	3.72 ± 0.91
Post-chilling rewarming	4	11.53 ± 3.50	3.22 ± 0.65	3.50 ± 0.40	3.96 ± 0.41
Chilling	6	14.68 ± 0.60	2.81 ± 0.19	4.42 ± 0.53	3.08 ± 0.45
Post-chilling rewarming	4	9.37 ± 0.82	7.43 ± 1.74	4.58 ± 1.01	5.46 ± 1.30

Table 3. Changes in the proportion of major fatty acids in acyl lipids upon chilling and rewarming of maize seedlings. Seedlings were chilled in the dark for 6 days and then rewarmed for 4 days in the light. Change in the proportion of fatty acids during chilling plus rewarming is expressed as an average (n=3) increase (+Δ) or decrease (-Δ) in relation to its proportion before chilling.

Lipid class	Breeding line		16:0	16:1 (*t*)	18:2	18:3	Σ	
			mol %				+Δ	-Δ
PG	CS	CM 7	+9.19	-4.76	+1.31	-7.16	10.50	11.92
		Co 151	+7.07	-3.34	+0.24	-5.37	7.31	8.71
	CT	S 215	+5.32	-2.12	+1.35	-4.96	6.67	7.08
		EP 1	+2.91	-0.08	+1.27	-3.81	4.18	3.89
MGDG	CS	CM 7	+1.67		+0.73	-3.03	2.40	3.03
		Co 151	+1.60		-0.49	-1.87	1.60	2.36
	CT	S 215	+0.68		-0.26	-0.70	0.68	0.96
		EP 1	+0.73		-0.04	-0.83	0.73	0.87
DGDG	CS	CM 7	+2.95		+0.84	-4.20	3.79	4.20
		Co 151	+1.50		-0.20	-1.73	1.50	1.93
	CT	S 215	+1.36		-0.19	-1.91	1.36	2.10
		EP 1	+0.64		-0.49	-1.31	0.64	1.80
SQDG	CS	CM 7	+11.18		-0.88	-11.62	11.18	12.50
		Col 51	+9.32		-2.27	-8.44	9.32	10.71
	CT	S 215	+5.94		-0.41	-5.60	5.94	6.01
		EP 1	+4.89		-0.05	-4.85	4.89	4.90

However, upon rewarming of seedlings UR in MGDG decreased by about 70 % and 45 % in CS and CT inbred, respectively, indicating more effective peroxidation of MGDG in these two groups. On the contrary, there was very low increase of UR in DGDG during chilling with the exception of some increase in the S 215 line. Upon rewarming a decrease of UR in DGDG was observed in all four inbreeds. Relatively constant UR was noticed in SQDG during chilling, but after rewarming, a decrease of this ratio was ranging between 40 % and 20 % in CS CM 7 line and CT EP 1 line, respectively, when compared to UR in non-chilled samples.

Proportion of htm fatty acids in PG and SQDG were not affected during chilling (Table 4). However, upon rewarming an increase in the proportion of htm fatty acids in PG and SQDG was greater in CS than in CT lines and more extensive in SQDG than in PG.

Double bound index

DBI in MGDG from control and chilled leaves of four inbred lines did not differ except in DGDG small increase was noted upon chilling (Table 5). However, after post-chilling rewarming DBI decreased in all four polar lipids, and again, more extensively in CS than CT lines. The same pattern of changes was observed for glycolipids and combined lipid classes.

Discussion

Successive action of LAH and LOX

Deacylation of galacto- and phospholipids during chilling and post-chilling rewarming of maize seedlings accompanied by release of FFA appears to be the consequence of low-temperature induced LAH activity. Hydrolytic breakdown of membrane lipids precede oxidative reactions since it was questioned the action of LOX on esterified fatty acids in plants tissues (Brown *et al.*, 1987; Fobel *et al.*, 1987). Deesterification of membrane phospholipids by free radicals without promoted peroxidation of unsaturated fatty acids (Senerata *et al.*, 1985) as well as free radical reaction with or without FFA addition results in degradation of phospholipids in model plant membrane (Barclay and McKersie, 1994) were reported. Quantitative relation between nonenzymic and enzymic deesterification of lipids in photosynthetic tissues is not established. Similarly, suggestion that a LOX form is capable of oxygenating linolenic acid residues of galactolipids in thylakoid membrane (Feussner and Wastermack,

Table 4. Changes in the fatty acid UR in polar lipids and in the proportion of htm fraction in both PG and SQDG during chilling and post-chilling rewarming. Values for individual parameters are calculated from fatty acid analysis on molar basis and are the means ± SE of 3 experiments

Parameter	Lipid class	Treatment	Days	Maize breeding line			
				CM 7	Co 151	S 215	EP 1
Unsaturation ratio [b]	MGDG	Chilling	0	101.60 ± 15.04	114.45 ± 9.64	119.99 ± 15.57	90.11 ± 14.47
			6	79.47 ± 5.52	92.36 ± 17.11	92.83 ± 12.88	80.18 ± 11.83
		Post-chilling rewarming [a]	4	34.43 ± 16.25	28.05 ± 1.30	57.97 ± 3.56	51.03 ± 6.62
	DGDD	Chilling	0	10.25 ± 0.54	10.02 ± 1.63	11.81 ± 0.52	7.78 ± 1.09
			6	11.45 ± 0.56	11.94 ± 0.79	14.14 ± 0.82	8.49 ± 0.30
		Post-chilling rewarming [a]	4	7.16 ± 1.05	7.89 ± 1.35	9.46 ± 0.38	7.06 ± 0.48
	SQDG	Chilling	0	1.81 ± 0.08	2.24 ± 0.14	2.54 ± 0.11	1.80 ± 0.09
			6	1.74 ± 0.08	2.21 ± 0.18	2.45 ± 0.03	1.69 ± 0.14
		Post-chilling rewarming [a]	4	1.11 ± 0.09	1.45 ± 0.23	1.89 ± 0.13	1.45 ± 0.11
htm-PG [c]	PG	Chilling	0	59.9 ± 1.7	58.1 ± 0.9	58.8 ± 1.3	59.7 ± 1.8
			6	57.7 ± 1.9	57.5 ± 1.0	57.9 ± 1.3	58.3 ± 1.2
		Post-chilling rewarming [a]	4	64.2 ± 4.2	62.4 ± 1.4	61.3 ± 1.0	60.9 ± 1.0
	SQDG	Chilling	0	36.0 ± 1.00	29.8 ± 1.9	28.4 ± 0.8	36.6 ± 1.7
			6	36.5 ± 1.0	31.2 ± 1.7	29.0 ± 0.2	37.1 ± 1.9
		Post-chilling rewarming [a]	4	47.5 ± 2.0	41.0 ± 2.1	34.6 ± 1.6	40.9 ± 1.6

[a] Rewarming for 4 days after 6 days of chilling
[b] Unsaturated ratio (UR) was calculated considering 16:1 (*t*) as saturated. UR = 16:1 (*c*) + 18:1 + 18:2 + 18:3 / 16:0 + 16:1 (*t*) +18:0
[c] Sum of high-temperature melting fatty acids (mol %) E htm FA = 16:0 + 16:1(*t*) + 18:0

1998) is, however, not supported by quantitative data indicating the extent of this reaction *in vivo*.

Free, but not esterified polyunsaturated fatty acids are substrates for lipoxygenase (LOX) the key enzyme initiating oxidative degradation of FFA. Most LOXs clearly prefer free polyunsaturated 18:3 and 18:2 acids and show little reactivity towards esterified fatty acids (Hildebrandt, 1989). LOX was detected in both stroma and thylakoid chloroplasts (Douillard and Bergeron, 1981; Bowsher *et al.*, 1992). The enzyme is likely to contribute to oxidative injury during chilling, ageing and senescence by initiating the chain reaction of lipid peroxidation and activated oxygen formation (Lynch and Thompson, 1984; Thompson *et al.*, 1987). It has been suggested that timing and extent of peroxidative reactions initiated by the availability of substrate i.e. FFA for the enzyme rather than changes in quantity of the enzyme *per se* is of importance (Thompson *et al.*, 1987). Thus, consecutive action of LAH which generates FFA followed by the action of LOX to produce $O_2^{\cdot-}$ and hydroxyperoxides appear to be initiated steps of the secondary events involved in chilling response.

Free fatty acid level

Reliable data about occurrence and abundance of FFA in plants are plentiful and restricted mostly to spinach and bean chloroplasts. FFA are very mobile metabolites being products of membrane lipid degradation, as well as depending on experimental conditions, substrates for lipid resynthesis or/and peroxidation. In senescent barley leaf segments extensive degradation of galactolipids (equivalent of 500 nmoles of 18:3) was accompanied by a progressive loss of free 18:3, however, only from 40 to 2 nmoles during 3 days. Lack of concomitant appearance of a pool of FFA during the main period of galactolipid breakdown appeared to be resulting to an increase of the 18:2/18:3 molecular species of phosphatidylcholine (Gut and Matile, 1989).

Table 5. Double bound index of lipid classes from leaves of CS and CT maize inbred lines. DBI for glycolipids and DBI for combined lipid classes were calculated by summation of lipid class values, weighted by the proportion of the lipid class in the glycolipid and glycolipid plus PG fraction of lipid extract, respectively. DBI for PG was calculated considering *cis*-double bound only. Each value is the mean ± SE of 3 experiments.

Lipid class	Treatment	Days	Maize breeding line			
			CM 7	Co 151	S 215	EP 1
MGDG	Chilling	0	5.87 ± 0.02	5.84 ± 0.02	5.87 ± 0.02	5.88 ± 0.02
		6	5.88 ± 0.02	5.85 ± 0.04	5.87 ± 0.01	5.89 ± 0.03
	Post-chilling rewarming [a]	4	5.72 ± 0.10	5.71 ± 0.08	5.82 ± 0.01	5.82 ± 0.02
DGDG	Chilling	0	5.36 ± 0.04	5.28 ± 0.09	5.40 ± 0.02	5.19 ± 0.10
		6	5.43 ± 0.04	5.42 ± 0.12	5.52 ± 0.02	5.26 ± 0.02
	Post-chilling rewarming [a]	4	5.14 ± 0.11	5.16 ± 0.11	5.30 ± 0.04	5.12 ± 0.06
SQDG	Chilling	0	3.66 ± 0.05	3.86 ± 0.12	4.05 ± 0.07	3.67 ± 0.11
		6	3.63 ± 0.06	3.89 ± 0.19	4.06 ± 0.06	3.64 ± 0.14
	Post-chilling rewarming [a]	4	2.94 ± 0.13	3.28 ± 0.26	3.69 ± 0.10	3.37 ± 0.11
PG	Chilling	0	2.28 ± 0.08	2.29 ± 0.06	2.39 ± 0.02	2.34 ± 0.09
		6	2.37 ± 0.11	2.33 ± 0.09	2.41 ± 0.08	2.37 ± 0.06
	Post-chilling rewarming [a]	4	1.92± 0.15	1.99 ± 0.09	2.15 ± 0.05	2.17 ±0.06
Glycolipids	Chilling	0	5.42 ± 0.03	5.44 ± 0.05	5.55 ± 0.01	5.44 ± 0.06
		6	5.44 ± 0.03	5.42 ± 0.06	5.54 ± 0.04	5.43 ± 0.03
	Post-chilling rewarming [a]	4	5.10 ± 0.11	5.09 ± 0.10	5.43 ± 0.04	5.35 ± 0.03
Combined lipid classes	Chilling	0	5.13 ± 0.02	5.11 ± 0.06	5.15 ± 0.01	5.10 ± 0.05
		6	5.08 ± 0.02	5.04 ± 0.09	5.14 ± 0.05	5.06 ± 0.02
	Post-chilling rewarming [a]	4	4.71 ± 0.12	4.73 ± 0.08	5.02 ± 0.04	4.97 ± 0.02

[a] Rewarming for 4 days after 6 days of chilling

FFA content in control leaves of three inbreeds was in the range of 0.16 - 0.30 while in CM 7 line it was 0.62 μmol·mg^{-1} Chl, respectively (Table 1). These two values appear to be reliable because they were obtained upon treatment of leaves with liquid nitrogen in order to inactivate lipolytic enzymes. Similarly, relatively low level of FFA was reported for chloroplasts of cucumber (Sączyńska *et al.*, 1993) as well as for CR wheat, pea, spinach and CT maize S 72 line (Sączyńska *et al.*, 1994b). Our data, as well as those of several authors for bean and spinach, are about 10-fold higher than those reported for spinach and horse bean by Percival *et al.* (1980). Reasons of these discrepancies were not, however, evaluated. The level of FFA higher than 0.30 μmol mg^{-1} Chl was found in chloroplasts of more chilling susceptible CM 7 line (Table 1) as well as in *L. esculentum* (Gemel *et al.*, 1988), maize F7Rp$_{III}$ line (Sączyńska *et al.*, 1994a), melon (Gemel and Kaniuga, 1989), bean and potato (Sączyńska *et al.*, 1994b).

During dark chilling of detached leaves of CS species for a few days results in 1.5 to 3-fold increase of FFA content in chloroplasts. The magnitude of this increase depends on the activity of LAH as shown for tomato (Gemel *et al.*, 1988), maize (Gemel *et al.*, 1989), and potato (Gemel and Kaniuga, 1989). Contrary, in dark chilled maize seedlings treated up to 6 days, changes in the FFA level were very small, except in chilling susceptible CM 7 line (Table 1). Thus it is likely that either induction of LAH activity in growing plants during chilling stress in dark or peroxidation or both is less effective than in detached leaves.

On the other hand, although rewarming of maize seedlings in the light subsequently to chilling for 6 days resulted in a pronounced degradation of polar

lipids (Kaniuga *et al.*, 1999b) correspondingly an increase of FFA level was not, however, observed (Table 2). Some accumulation of FFA was noticed in Co 151 and EP 1 lines, only. In contrast in CM 7 line the level of FFA decreased while in line S 215 it remained constant. This pattern of FFA changes seems to be caused by various activities of oxidative systems involved in FFA catabolism as recently reported for individual maize inbreeds by Hodges *et al.* (1996, 1997).

It is interesting that in chloroplasts of CS closely related cultivars or inbred lines of the same age the original level of FFA may differ greatly, by the factor 2.5, 3 and 6 in tomato (Gemel *et al.*, 1988), both melon (Gemel and Kaniuga, 1989) and red pepper (Sączyńska *et al.*, 1990) and cucumber (Sączyńska *et al.*, 1993), respectively. Similar level of FFA was found in leaves of CS and CT potato species but it was different upon chilling (Gemel and Kaniuga, 1989). Following analysis of data for chloroplast FFA content two pools of FFA were recognized (Sączyńska *et al.*, 1993). The first one is originally exhibited in freshly isolated chloroplast and which does not affect PSII activity. The second one is generated in CS species during chilling of detached leaves or ageing of chloroplasts and is correlated with the action of galactolipase and inactivation of PSII activity (Kaniuga *et al.*, 1978; Percival *et al.*, 1980, Sączyńska *et al.*, 1993).

Fatty acid peroxidation

The peroxidation reactions that occur in photosynthetic tissues do not follow the classical reactions which have been detailed so well in model systems or microsomal membranes. Although contribution of lipid peroxidation to chilling injury appear to be largely elucidated (*cf.* Parkin and Kuo, 1989; Hariyadi and Parkin, 1993; Parkin *et al.*, 1989) there were some unclear points in respect to e.g. contribution of esterified fatty acids to peroxidation as well as participation of endo- and exogenous factors stimulating or inhibiting peroxidation in leaf segments or chloroplasts.

Relatively steady-state levels of FFA in the thylakoids of cucumber seedlings (Hariyadi and Parkin, 1993) and maize chilled and rewarmed seedlings (Table 1 and 2) imply that FFA are either rapidly consumed in oxidative processes, preventing their accumulation or esterified fatty acids are sensitive to peroxidation. If the concept that esterified fatty acids might be oxidized in peroxidizing membranes (Porter, 1984) or lipid fluorescent pigments could be formed from esterified acids in peroxidizing model membranes (Iio and Yoden, 1988) was valid, one would expect that extensive peroxidation could proceed during chilling without delay, because the level of esterified fatty acid as substrate would be not a limiting factor. This is, however, not the case. Peroxidation of thylakoids lipids in cucumber seedlings was induced during the rewarming period, after only 1 to 2 days of chilling (Hariyadi and Parkin, 1993) and in cucumber fruit after 3-7 days (Parkin and Kuo, 1989) and it was more extensive in maize seedlings after 6 days (Table 4) than upon 4 days of chilling (data not shown).

The magnitude of changes in both fatty acid unsaturation ratio (UR) in MGDG, DGDG and SQDG (Table 4) and double bond index (DBI) in glycolipids and combined lipid classes (Table 5) especially after post-chilling rewarming reflects the extent of lipid peroxidation. Fatty acid UR decreased upon post-chilling rewarming of cucumber fruit (Parkin and Kuo, 1989) and tomato fruit (Whitaker, 1994) but increased in bell pepper during chilling and subsequent rewarming (Whitaker, 1995). In maize seedlings selective reduction of fatty acid UR in MGDG by about 10 to 25 % during chilling for 6 days at relatively stable fatty acids UR in both DGDG and SQDG (Table 4) appears to corelate with the extent of MGDG and other polar lipids breakdown by LAH (Kaniuga *et al.*, 1999b) rather than with non-enzymic deesterification of MGDG followed by fatty acids peroxidation. It was recently found that peroxidation proceeds also in the absence of light and its extent depends on the original FFA level in CS and CR species or on its enhancement in thylakoids by lipase or detergent treatment (Garstka *et al.*, 1994). Further, upon post-chilling rewarming decrease of fatty acid UR by about 50 % and 70 % in CT and CS inbreeds, respectively, relative to non-chilled control was noted. Differences between CS and CT lines in the extent of UR decrease (Table 5) at similar fatty acid composition of polar lipids in all four inbreeds (Kaniuga *et al.*, 1999a) may be related to more ef-

fective hydrolysis of polar lipids in CS than in CT lines (Kaniuga *et al.*, 1999b). Therefore, it is likely that non-enzymic peroxidation of fatty acids does not play an essential role *in vivo*.

Contribution of SOD to chilling stress

The balance between the formation and detoxification of activated oxygen species by antioxydant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase which eliminate toxic oxygen byproducts appeared to be critical to cell survival during periods of stress such as chilling (*cf.* Bowler *et al.*, 1992). An increased resistance to oxidation stress was found in transgenic plants that overexpress chloroplastic Cu/Zn-superoxide dismutase (Sen Gupta *et al.*, 1993a) or upon combine increase of Cu/Zn-superoxide dismutase and ascorbate peroxidase (Sen Gupta *et al.*, 1993b) as well as upon expression of Mn-superoxide dismutase transgen in transgenic alfalfa (McKersie *et al.*, 1993).

However, in several original plant species relations between SOD activity and chilling response were less obvious. Neither significant differences in the level of SOD activity between CS cucumber and oleander grown at 45 °C and CR spinach and oleander grown at 20 °C were found, nor was there any significant change in SOD activity when slices were incubated at chilling temperature (Hodgson and Raison, 1991b). Similarly, the activities of SOD, ascorbate peroxidase and monodehydroascorbate reductase were very similar in *L. hirsutum* and *L. esculentum*, which differ in their chilling sensitivity and did not increase in response to chilling, while a higher gluthatione reductase activity was only observed (Walker and McKersie, 1993). It is also interesting, that in four inbred lines of maize, differing in chilling sensitivity, SOD activity did not follow response of other antioxidant enzymes to chilling stress (Hodges *et al.*, 1997).

Reactive oxygen species and peroxidation in photosynthetic tissues

Involvement of reactive oxygen species in initiation/stimulation of lipid peroxidation is commonly accepted as an important factor, but recent data concerning this subject in respect to photosynthetic tissues are not consistent. Thus, chilling of leaf slices at moderate light at 5 °C of CS cucumber and oleander grown at 45 °C stimulated $O_2^{\cdot-}$ production in thylakoids (Hodgson and Raison, 1991a). However, it did not increase peroxidation of membrane lipids in these plants (Hodgson and Raison, 1991b) while a pronounced stimulation of ethane production in chilled cucumber leaf segment at high light was observed (Wise and Naylor, 1987).

Further, there is a controversy concerning both a limited peroxidation in CR plants and the stimulation of peroxidation in these plants by methyl viologen (MV), a well-known effector of $O_2^{\cdot-}$ production. In pea leaves ethane production was not observed in the cold or in the light for 6 hour, until leaves were pretreated with MV. Then, ethane production began and continued at the same rate as in chilled cucumber in the absence or presence of MV (Wise and Naylor, 1987). The question arises why does MV stimulate peroxidation only in pea and not in cucumber ? On the contrary, Hodgson and Raison (1991b) observed inhibition of peroxidation in chilled cucumber leaf slices by MV while in samples chilled in the light and incubated in the dark a two fold increase of malondialdehyde formation was noted. Moreover, in the presence of Rose Bengal, producing singlet oxygen, malondialdehyde production was more than twofold stimulated in both CR spinach and CS cucumber (Hodgson and Raison, 1991b). These differences between the extent of peroxidation in CS and CR species were attributed to the various activites of the antioxydant enzyme systems for removal of toxic oxygen species prior to peroxidation in CS and CR plants (Wise and Naylor,1987; Hodgson and Raison, 1991b).

There is also no agreement about the effect of temperature on the peroxidation. Low temperature (5 °C) was almost 2-4-fold more effective than 25 °C in stimulation of peroxidation in cucumber leaf segments (van Hasselt, 1974; Wise and Naylor, 1987), contrary to data for cucumber, oleander and spinach leaf slices in which malondialdehyde formation at 4 °C was about 15 % lower than in slices incubated for the same time at 25 °C (Hodgson and Raison, 1991b). All these discrepancies seem to indicate a complex mechanism of lipid peroxidation in photosynthetic tissues of CS and CT species. Some of these discrepancies may be related to various levels

of FFA in chloroplasts of CS and CT plants due to the various activity of galactolipase.

Accessibility of FFA affects response to chilling stress

If we assume in line with the recent concept that oxidative stress is responsible for evocation of chilling injury (*cf.* Parkin *et al.* 1989, Hariyadi and Parkin, 1993) availability of FFA as substrate for peroxidation appears to play an important role. Abundance of FFA results mostly from the action of LAH while reliable content of FFA in tissue depends on the balance between the formation of FFA and their oxidative catabolism. The lower level of FFA in maize seedlings upon chilling and rewarming (Table 1 and 2) than upon chilling of detached leaves of CS species (Gemel *et al.* 1988, 1989) may be related to more effective peroxidation stimulated by light during post-chilling rewarming of seedlings, whereas the extent of FFA release by LAH during chilling in the dark of both detached leaves or growing seedlings seems to be similar.

Conclusions

Our comparative studies on the effect of low-temperature stress on maize seedling varying in chilling susceptibility indicated that the enhanced polar lipid degradation by LAH during post-chilling rewarming followed by peroxidation of fatty acids are responsible for different response to chilling of CS and CT maize genotypes.

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