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COMPOSITION OF NON-ESTERIFIED FATTY ACIDS IN CHLOROPLASTS OF CLOSELY RELATED CHILL-SENSITIVE PLANTS*

JOANNA GEMEL, VIOLETTA SACZYŃSKA and ZBIGNIEW KANIUGA†

Institute of Biochemistry, University of Warsaw, Al. Żwirki i Wigury 93, 02–089 Warszawa, Poland

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Key Word Index—*Cucumis sativus, C. melo; Lycopersicon esculentum, L. hirsutum, L. peruvianum; Passiflora edulis; Solanum tuberosum, S. chaucha, S. ajanhuirii, S. toralapanum; Zea mays*; Cucurbitaceae; Passifloraceae; Solanaceae; chloroplast; non-esterified fatty acids.

Abstract—The non-esterified fatty acid (NEFA) composition of chloroplasts of 19 closely related chill-sensitive species of cucumber, melon, maize, passion fruit, wild potato and tomato with different chill tolerances was studied. Chloroplasts of chill-tolerant (CT) and chill-sensitive (CS) plants contain an average NEFA level of 0.30 and 0.65 μmol NEFA/mg Chl, respectively with the exception of potato which has the same content of NEFA in both CT and CS species. Unsaturated C_{18} fatty acids comprise 73.8% of the total NEFA with the most abundant acid, 18:3, ranging from 45.6 to 83.5% of the total NEFA, 16:0 is the second most abundant fatty acid with an average of 13.9% of the total NEFA. There are some discrepancies in the content of individual fatty acids from the general pattern of NEFA composition in all species studied, 20:0 is present in potato only, while 16:2 occurs in tomato and some forms of melon. A low proportion of 18:3 is found in melon (line 190) and *L. hirsutum* (3 100 m) whereas a relatively high level of 18:1 is present in potato. The content of individual C_{18} and C_{16} fatty acids in some tomato and melon species is higher or lower than in the other plants. It is rather difficult to correlate the double bond index with the degree of chill sensitivity of the plants used. It is concluded that the content of NEFA in chloroplasts may be useful parameter for evaluation of chill sensitivity of closely related species.

INTRODUCTION

Although the lipid composition of the chloroplast membrane is well documented [cf. 1], our knowledge about the non-esterified fatty acid composition of chloroplasts is rather poor. It is known, however, that both endo- and exogenous non-esterified fatty acids (NEFA) even at low concentrations have deletorious effects on the structure and function of chloroplasts. Exogenous NEFA, especially 18:3 are known to affect the photochemical activities [2, 3] and the ultrastructure of chloroplasts [4, 5] and cause Mn depletion [6]. Ultrastructural changes caused by endogenous fatty acids were observed following ageing of chloroplasts [7], digestion of chloroplasts with bean galactolipase [8] or cold treatment of leaves of chill-sensitive (CS) plants [9]. This latter treatment results in loss of Hill reaction activity [10], accumulation of NEFA in chloroplasts [11] and release of Mn [6].

Recently we have found a distinct content of NEFA in chloroplasts of CS and chill-resistant (CR) plants [12, 13]. In this paper, we report on the composition and level of NEFA in chloroplasts of closely related CS plants but with different tolerances towards chilling. The results seem to indicate that, in contrast to NEFA composition, the level of NEFA is a characteristic parameter of the chill sensitivity of these plants.

*Part 23 of the series 'Photosynthetic apparatus of chilling-sensitive plants'. For previous Parts concerning this subject see refs [11–13].

†Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Chilling sensitivity as measured by the extent of Photosystem II inactivation following cold treatment of leaves [10] is shown in Table 1. In all of the more chill-tolerant (CT) plant species tested the residual activity is much higher than in the chill-sensitive (CS) plants.

The distribution and abundance of NEFA in chloroplasts of 10 CT and 11 CS species is shown in Table 2. The content of total NEFA in chloroplasts of the CT species is lower than in the CS ones ranging from 0.12 to 0.44 and from 0.34 to 1.17 μmol NEFA/mg Chl, respectively. The mean values for these two groups of plants were 0.30 and 0.65 μmol NEFA/mg Chl, respectively.

The composition of NEFA in chloroplasts (Table 2) is essentially comparable with the fatty acid composition of leaves of four CS plants [14] and cucumber chloroplasts [15], although there are several deviations from the general pattern. The most abundant acid is 18:3 which accounts for 44–83% of the total NEFA with the exception of *L. hirsutum* (3100 m) in which the very low content (26.3%) of this fatty acid is compensated for by the high level of 18:0 (39.3%). It is interesting to note that, in contrast to the rest of plants studied, potato chloroplasts contain a high proportion of 18:1 (12.8–19.0%). In addition, 20:0 was found in this plant only. The level of 18:2 is roughly equal (3.5–11.7% of the total NEFA) in all plants except for *L. hirsutum* (700 m) and *L. peruvianum* which contain 17.0 and 29.1% of the total NEFA, respectively.

The major saturated fatty acid is 16:0. Its content is low (5.7–9.5% of the total NEFA) in cucumber line 303,

Table 1. Chill sensitivity test

Type*	Plant	Days of† chilling	Photochemical activity of control‡	Residual activity (%)
	Cucumber			
CS	Warszawski	2	44	7
	Skierniewicki	2	39	12
CT	Line 303	2	48	61
	Borszczagowski	2	48	72
	Melon			
CS	Hearts of Gold	1	75	45
	Line 191	1	33	60
CT	Line 14	1	65	102
	Line 190	1	123	100
	Maize			
CS	Line F7 Rp III	3	49	0
CT	Line S72	3	87	76
CT	*Passiflora edulis*	4	25	100
	Potato			
CS	*S. tuberosum*	6	95	16
	S. toralapanum	6	115	35
CT	*S. ajanhuirii*	6	128	70
	S. chaucha	6	104	86
	Tomato			
CS	*L. esculentum*	4	100	22
	L. hirsutum (700 m)	4	86	47
CT	*L. hirsutum* (3100 m)	4	48	60
	L. peruvianum	4	79	72

*CT and CS denote chill tolerant and chill-sensitive plants.

†Leaves of cucumber and melon were chilled at 4.5° while other plants were chilled at 0°.

‡μmol of O_2 evolution hr mg Chl; for tomato μmol DCIP reduced/hr/mg Chl. Data are means of three experiments.

melon line 191 and 'Hearts of Gold' as well as in *S. tuberosum* and *L. hirsutum* (3100 m) but a high content (26.3% of the total NEFA) has been found in melon line 190, similar to that of 16:1 (15%) in this latter species.

The double bond index (DBI) is not, however, correlated with the chill sensitivity. This is to be expected since previous studies indicated that the differences in fatty acid composition in lipids of either thylakoid membranes [16] or whole leaf lipids [14] in CS and CR plants as well as in leaves of both CS and CR alfalfa [17] and *Passiflora* species [18] are small and insufficient to discriminate between CS and CR plants. A higher DBI in chloroplast membranes of CR cultivars of alfalfa than in those of CS ones was found by Peoples *et al.* [19] only.

The level of NEFA in chloroplasts of expanded leaves appeared to be relatively stable for several weeks during the middle period of plant vegetation [20]. The differences in the content of NEFA in chloroplasts of CS and CR plants [13] are now observed for closly related CS species with different chill sensitivity. Thus, the NEFA level is lower in CT than in CS species (Table 2). This finding is in agreement with a higher galactolipase activity in chloroplasts of the latter group [13, 21]. The relationship between the level of galactolipase activity and NEFA in chloroplasts of closely related plants is also clearly evident as a result of the much faster accumulation of NEFA in chloroplasts following chilling of more susceptible species than of those of greater chill tolerance [21]. The present data seem to indicate that the level of NEFA in chloroplasts may be a useful parameter for determination of the chill sensitivity of closely related plant species. Whether the same relationship will be observed for these plants in the case of leaf NEFA levels remains to be shown.

EXPERIMENTAL

Plants. Varieties and lines of cucumber, melon, wild potato and domestic tomato variety Norton were cultivated under greenhouse conditions, while maize and wild tomatoes were grown under field conditions. The mean altitudes of wild tomatoes originally growing at low and high altitudes in Peru are given in parentheses. In all experiments young, fully expanded leaves were used. Differences in chill sensitivity of plants used in the present work were known either from horticultural practice, breeding selection or literature and were additionally checked by the extent of Photosystem II inactivation following chilling stress

Table 2. Non-esterified fatty acid composition in chloroplasts of closely related chill-sensitive species*

Type†	Plant	n	Total NEFA (μmol/mg Chl)	% total 16:0	16:1	16:2	18:0	18:1	18:2	18:3	20:0	$\Sigma_{C_{18}}$	DBI
	Cucumber												
CS	Warszawski	3	1.17±0.01	13.7±0.8	5.5±0.5	nd	3.1±0.3	3.4±0.3	6.7±0.5	69.0±0.3	nd	79.1	2.29
	Skierniewicki	3	0.94±0.02	15.1±1.3	5.9±0.6	nd	3.8±0.4	2.5±0.3	7.3±0.7	65.1±3.2	nd	74.9	2.18
CT	Line 303	3	0.44±0.01	7.2±0.8	5.2±0.5	nd	1.3±0.1	2.4±0.5	4.4±0.8	79.7±1.5	nd	86.5	2.56
	Borszczagowski	3	0.44±0.01	16.9±2.7	7.1±0.5	nd	2.6±0.2	3.6±0.3	7.4±0.8	61.6±2.6	nd	72.6	2.10
	Melon												
CS	'Hearts of Gold'	4	0.66±0.02	4.9±0.7	2.9±0.6	2.7±0.4	1.7±0.3	2.4±0.1	5.3±0.4	79.8±1.6	nd	87.5	2.61
	Line 191	3	0.85±0.01	7.6±0.4	3.1±0.3	nd	1.0±0.1	1.4±0.2	3.5±0.1	83.5±0.5	nd	88.4	2.62
CT	Line 14	4	0.17±0.02	17.2±2.3	3.3±0.9	3.3±0.4	7.8±1.1	5.5±0.9	7.3±0.3	56.0±5.9	nd	68.8	1.98
	Line 190	3	0.12±0.02	26.3±3.3	15.0±1.7	nd	6.3±0.2	7.7±0.8	7.6±0.6	38.5±1.7	nd	53.8	2.16
	Maize												
CS	Line F7 Rp III	7	0.43±0.02	16.8±1.3	6.8±0.6	nd	2.1±0.1	2.7±0.4	5.4±0.1	67.1±0.7	tr	75.2	2.24
CT	Line S72	4	0.17±0.01	24.5±3.9	6.4±0.6	nd	5.4±0.4	4.4±0.7	6.7±0.3	47.0±4.2	5.6±1.2	58.1	2.58
CT	*Passiflora edulis*	1	0.23	12.4	5.1	5.9	8.9	7.9	15.8	44.0	nd	67.7	1.88
	Potato												
CS	*S. tuberosum*	3	0.34±0.01	5.7±0.5	1.1±0.2	nd	1.0±0.1	19.0±1.4	7.9±0.5	63.4±1.2	1.6±0.2	90.3	2.26
	S. toralapanum	4	0.39±0.02	18.6±1.6	5.4±0.7	nd	3.2±0.2	13.5±0.7	11.7±0.8	44.2±4.3	3.9±0.7	69.4	1.75
CT	*S. ajanhuirii*	3	0.36±0.02	9.5±0.3	2.6±0.3	nd	0.8±0.1	16.2±1.0	8.3±0.9	63.1±0.7	nd	87.6	2.25
	S. chaucha	4	0.39±0.01	13.4±0.4	5.5±0.2	nd	3.5±0.2	12.8±1.9	10.8±1.3	50.8±2.4	2.7−0.2	74.4	1.92
	Tomato												
CS	*L. esculentum*	3	0.53±0.02	17.1±0.7	6.1±0.5	tr	1.5±0.2	6.1±0.2	9.7±0.3	60.0±1.2	nd	75.8	2.12
	L. hirsutum (700 m)	3	0.53±0.02	16.1±1.2	4.6±0.7	2.5±0.2	6.4±0.6	6.1±0.4	17.0±0.7	47.1±2.9	nd	71.2	1.91
CT	*L. hirsutum* (3100 m)	3	0.42±0.02	8.8±1.6	4.1±0.5	4.9±0.9	39.5±3.6	7.5±1.6	8.8±1.3	26.3±2.3	nd	42.6	1.17
	L. peruvianum	3	0.27±0.01	11.5±0.4	3.5±1.2	tr	5.0±1.6	5.3±1.1	29.1±5.1	45.6±4.9	nd	80.0	2.04
	Mean for CT species		0.30 μmol NEFA/mg Chl										
	Mean for CS species		0.65 μmol NEFA/mg Chl										
												73.8%‡	

*Values are means±s.e. of *n* experiments.
†CT and CS denote chill tolerant and chill-sensitive species.
‡Mean of the sum of unsaturated C_{18} as % of the total NEFA.
nd: Not detected.
tr: trace.

of leaves [10]. Decrease in Hill reaction activity or oxygen evolution was measured [6].

Isolation of chloroplasts. Chloroplasts were isolated from leaves as described previously [13]. Isolated chloroplasts were mostly of type B (according to the classification of Reeves and Hall [22]) accompanied by a small portion of the type C as judged from their appearance under phase contrast microscopy. Chlorophyll was determined according to Arnon [23].

Determination of non-esterified fatty acids. Immediately after isolation of chloroplasts, a sample (equivalent to 3–4 mg Chl) was treated with acid ethanol to stop lipid hydrolysis [24] and the fatty acids were extracted with hexane. The constituents of the hexane extract were converted to Me esters using CH_2N_2 reagent. The esters were purified by TLC on silica gel using hexane–Et_2O–HOAc (70:30:1). After removing the solvent, the lipid bands were detected by brief exposure to I_2 vapour. The bands of fatty acid Me esters were scraped off the plates, eluted from the silica gel with Et_2O and dried. Fatty acid Me esters were separated and identified by GC (glass column, 1 m × 3 mm i.d., 15% EGSS-X on Gas-Chrom P, 100–120 mesh, column temp. 178°, H_2 FID oven temp. 198°, N_2 40 ml/min, Me ester of behenic acid used as internal standard). The column was standarized with Me esters of pure fatty acids. The compounds were identified by comparing their R_fs with those of pure Me esters. The samples were stored under N_2 until analysis. The peak areas were measured as hight × peak width at half-peak height. Each sample was run with two parallel samples.

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