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Plant Biochemistry

Effect of Geographic Origin of Sea Buckthorn on the Triacylglycerol Composition of its Fruits

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(Presented by Academy Member T. Beridze)

ABSTRACT. Separate geographic forms of sea buckthorn (*Hippophae rhamnoides* L.) are characterized not only by different composition of triacylglycerols (TAGs) of their fruit mesocarp oil, but also by a different biosynthetic pattern of the TAGs. Siberian, Central Asian, and Baltic forms contain TAGs, which include mostly hexadecenoic ($C_{16:1}$) and palmitic ($C_{16:0}$) fatty acid (FA) residues and are synthesized according to the theory of 1,3-Random, 2-Random Distribution of FAs between the respective OH groups of glycerol. At the same time, the mechanism of formation of TAGs of the Caucasian form rich in the residues of $C_{16:0}$ and octadecenoic acid ($C_{18:1}$) is in accordance with the theory of Restricted Random Distribution of FAs. The occurrence of two different patterns of TAG biosynthesis in the mesocarp of the same biological object is demonstrated here for the first time. As for seed oils, the geographic forms studied here virtually do not differ from each other in the FA composition of their TAGs. This paper is dedicated to Professor Guivi Sanadze on the occasion of his 80th birthday. *© 2010 Bull. Georg. Natl. Acad. Sci.*

Key words: *$[A]_{1,2,3}$, $[A]_2$, and $[A]_{1,3}$ = the content of a fatty acid "A" in the* sn-1,2,3, sn-2, *and* sn-1,3 *positions of TAGs, respectively (mol %), $[A]_{2calc.}$ = the "A" content in the* sn-2 *position of TAGs (mol %) calculated from the $[A]_{1,2,3}$ data, FA = fatty acid, H = hexadecenoic acid (sum of positional isomers), KAS II =* 3-*ketoacyl-acyl carrier protein synthase II, L = linoleic acid, LPAAT = lysophosphatidic acid acyltransferase, O, octadecenoic acid (sum of positional isomers), P = palmitic acid,* P *= confidence limit of correlation, r = correlation coefficient, S and U = total saturated and total unsaturated FAs, TAG = triacylglycerol, TLC = thin-layer chromatography.*

Introduction

Previously, we have determined the composition of triacylglycerols (TAGs) of fruit mesocarp (hypanthium) oil of three cultivars of sea buckthorn (Ozerinina et al., 1988). The structure of the TAGs has been shown to conform to the theory of 1,3-Random, 2-Random Distribution of fatty acid (FA) residues in TAG molecules (Gunstone, 1962; Litchfield, 1972; Vereshchagin, 1972).

All of the cultivars studied by us up to now belong to a Siberian geographic form of sea buckthorn. However, throughout the former Soviet Union area, there are a number of other geographic forms of this plant, *viz.* Central Asian, Baltic and Caucasian ones, which were formed under the influence of various growth conditions and, possibly, different genotypic factors (Eliseev, 1982). Earlier, we have pointed out that these forms are characterized by a significant variability in the FA composition of mesocarp oil (Berezhnaya et al., 1993). Thus, Siberian and Central Asian plants are distinguished by the prevalence of hexadecenoic acid (Zham'yansan, 1978;

Zhmyrko et al., 1978, 1984, 1987; Loskutova et al., 1989). At the same time, TAGs of the Caucasian form are rich in octadecenoic acid and saturated (S) acids, and simultaneously are marked by a lower concentration of hexadecenoic acid (Mamedov et al., 1981, 1984; Murav'ev et al., 1985; Aslanov and Mamedov, 1986). The Baltic form is yet to be investigated in this respect.

Sea buckthorn fruit mesocarp oil is made up almost exclusively of TAGs, and, therefore, the differences between the plants of various forms in the total FA composition of the oil reflect first of all different qualitative and quantitative compositions of reserve TAGs themselves (Berezhnaya et al., 1988, 1993; Ozerinina et al., 1988). Therefore, the investigation of the molecular species composition of TAGs of plants of all geographic forms and of the patterns of TAG formation would be of significant interest. Nevertheless, respective data are scarce and fragmentary (Mamedov et al., 1981; Zhmyrko et al., 1984, 1987).

The subject of the present work was the comparative investigation of Central Asian, Baltic, and Caucasian geographic forms as regards the composition, structure, and formation pattern of their mesocarp TAGs as well as the comparison of the results obtained with the respective data for the Siberian form published earlier (Ozerinina et al., 1988).

Materials and Methods

Mature fruits of sea buckthorn (*Hippophae rhamnoides* L.) of Central Asian, Caucasian, and Baltic forms were collected during the same summer season in the flood-plains of the Pyandzh (Tadzhikistan) and Chorokhi (Georgia) rivers as well as on the Baltic seashore (Baltic Spit, Donskoye settlement), respectively.

Fruits were fixed in boiling *iso*-propanol and separated into mesocarp and seeds; lipids of these fruit parts were extracted separately (Berezhnaya et al., 1988). TAGs were isolated from a total lipid mixture by preparative TLC, subjected to lipase (EC 3.1.1.3) hydrolysis, and the *sn*-2 monoacylglycerols separated by TLC (Ozerinina et al., 1987). The FA composition of both TAGs and their hydrolysis products was established (Berezhnaya et al., 1988). The positional-species and molecular species compositions of TAGs were calculated on the basis of both experimental data and existing hypotheses of FA distribution in TAGs (Gunstone, 1962; Gunstone et al., 1965; Litchfield, 1972; Vereshchagin, 1972).

All experiments were performed in triplicate. Significance of the confidence limit of correlation (*P*) between the plants of various geographic forms in their actual FA and TAG composition as well as between an experimentally found TAG composition of a plant of a given form and a theoretically calculated one was determined by standard procedures, using respective correlation coefficients (*r*).

Results

Fatty acid composition of mesocarp triacylglycerols

Differences mentioned above among various geographic forms in the total FA composition of mesocarp TAGs ($[A]_{1,2,3}$ values) have not yet been proven to be statistically significant. Therefore, first of all, the $[A]_{1,2,3}$ data for the TAGs of the three geographic forms were obtained (Table 1). One can see that these forms are indeed different in the FA composition of their TAGs. In the plants of Central Asian and Baltic forms, as well as in Siberian ones (Ozerinina et al., 1988), hexadecenoic and palmitic acids are the major FAs. At the same time, in Caucasian sea buckthorn TAGs, octadecenoic acid predominates among all unsaturated FAs, and in their palmitic acid concentration they exceed the TAGs of all other geographic forms. Also noteworthy is the fact that the TAGs studied here (Table 1) are devoid of hexadecadienoic acid, which is present in an appreciable amount (2 - 4 %) in TAGs of the Siberian form (Berezhnaya et al., 1988; Ozerinina et al., 1988).

The $[A]_{1,2,3}$ values from Table 1 as well as the respective results for the Siberian form (Ozerinina et al., 1988) were subjected to correlation analysis (Table 2). It follows from Table 2 (values outside of brackets) that, as regards FA composition of fruit mesocarp TAGs, the Caucasian form is significantly different from other ones, which as a whole are similar to one another in this respect. The data obtained suggest that, in this regard, all geographic forms investigated here can be divided into two groups: Siberian, Central Asian, and Baltic ones belong to Group 1, and Caucasian, to Group 2.

Concentrations of individual FAs in the mid- ($[A]_2$) and extreme ($[A]_{1,3}$) positions of sea buckthorn fruit mesocarp TAG molecules are also presented in Table 1. One can see that the *sn*-2 position of all TAGs studied here includes mainly monoenoic acids. As for palmitic acid, it is almost exclusively concentrated in the extreme positions of TAGs of Central Asian and Baltic plants; however, TAGs of the Caucasian form are characterized by a significant amount of palmitate in the *sn*-2 position as well (11.8 mol %).

Moreover, the values of $[A]_{1,2,3}$, $[A]_2$, and $[A]_{1,3}$ in Table 1 demonstrate that Baltic sea buckthorn, while falling into Group 1, is nevertheless in most cases intermediate between Central Asian and Caucasian forms both in the concentration of individual monoenoic FA spe-

Table 1

Fatty acid composition of the *sn*-1,2,3, *sn*-2, and *sn*-1,3 positions of fruit mesocarp triacylglycerols of various geographic forms of sea buckthorn.[a]

Geographic forms	Position in triacyl-glycerols	Fatty acid composition, mol %					
		P	H	O	L	Total C_{16}	Total C_{18}
Central Asian	*sn*-1,2,3	34.2±1.0	54.8±1.6	11.0±0.2	0.0	89.0	11.0
	sn-2	0.6±0.2	80.3±2.5	19.1±1.6	0.0	80.9	19.1
	sn-1,3	51.0±1.0	42.0±3.0	7.0±1.6	0.0	93.0	7.0
	sn-$2_{calc.}$	2.0	83.7	14.3	0.0	85.7	14.3
Baltic	*sn*-1,2,3	32.6±1.2	42.4±0.9	20.8±1.2	3.5±0.8	75.0	24.3
	sn-2	2.1±0.6	53.6±2.1	34.9±1.1	7.8±0.6	55.7	42.7
	sn-1,3	47.8±1.3	36.8±2.3	13.7±1.6	1.4±1.0	84.6	15.1
	sn-$2_{calc.}$	2.0	64.3	26.7	6.0	66.3	32.7
Caucasian	*sn*-1,2,3	47.0±1.7	16.4±0.7	35.0±1.0	1.6±0.5	63.4	36.6
	sn-2	11.8±1.0	19.5±0.9	65.4±1.2	3.3±0.6	31.3	68.7
	sn-1,3	64.6±2.0	14.9±1.1	19.8±1.5	0.7±0.	79.5	20.5

[a] The means of three analytical replications and their standard errors are presented. TAGs of Baltic form contain also linolenic acid; its concentration in the *sn*-1,2,3, *sn*-2, *sn*-1,3, and *sn*-$2_{calc.}$ positions is 0.7, 1.6, 0.3 and 1.0 mol %, respectively. For Central Asian and Baltic forms, correlation between $[A]_2$ values found experimentally and $[A]_{2calc.}$ calculated according to Litchfield is significant (r = 0.996 and 0.973, respectively; P > 99 %). $[A]_{2calc.}$ values for the Caucasian form were not calculated (see text).

cies in the respective positions of TAG molecules and in the relative content of total C_{16} FAs and total C_{18} FAs in these positions.

Finally, Table 1 includes not only FA concentrations in the *sn*-2 position of Central Asian and Baltic sea buckthorn TAGs found experimentally, but also those calculated using the equations of Litchfield ($[A]_{2calc.}$, see Ozerinina et al., 1987). These equations are based on the theory of 1,3-Random, 2-Random Distribution of FA residues between the respective positions of a TAG glycerol moiety (Litchfield, 1972). One can see that as regards Central Asian and Baltic sea buckthorn these results are as a whole consistent with the $[A]_2$ values found experimentally. For the Caucasian form, calculation of $[A]_{2calc.}$ values was impossible, because the saturated FA level in its TAGs ($[S]_{1,2,3}$ = 47 mol %) was more than 1.5 times higher than that allowed for Litchfield's equations, *i.e.* 30 % (Litchfield, 1972). As regards the extent of affinity of each unsaturated FA species to the *sn*-2 position of TAGs, the three geographic forms studied here are generally similar to the Siberian one (see Table 2 in Ozerinina et al., 1988).

Positional-species composition of mesocarp TAGs

Because of the evidence that various forms differ from each other both in the total FA composition and the positional distribution of FAs in mesocarp TAGs, and in order to draw a definite conclusion that there is a significant difference between Groups 1 and 2 in the TAG composition of their mesocarp oil, we compared them with each other in respect of the most differential category of TAG composition, *viz.* their positional-species composition. The latter represents concentrations (mol % of total TAGs) of TAG positional species, which are characterized, first, by specific individual FA composition, and, second, by a mid- (*sn*-2) or extreme (*sn*-1,3) position of the residues of these FAs in a TAG molecule (Vereshchagin, 1972).

Table 2

Coefficients of correlation (*r*) between various geographic forms of sea buckthorn as regards the fatty acid composition and the experimentally found positional-species composition (in brackets) of their fruit mesocarp triacylglycerols.[a]

Geographic forms	Siberian [c]	Central Asian	Caucasian
Central Asian	0.986 (0.955)	-	-
Baltic	0.921 (0.798)	0.963 (0.914)	0.644[b] (0.347[b])
Caucasian	0.473[b] (0.000[b])	0.266[b] (0.091[b])	-
Central Asian [d]	-	0.994 (0.967[f])	-
Caucasian [e]	-	-	0.999
Siberian [f]	0.900	-	-

[a] The number of pairs of measurements *n* for Siberian, Central Asian, Baltic and Caucasian forms is equal to 7, 4, 5, and 4 (number of fatty acid species), and to 24, 15, 21, and 20 (number of TAG species, in brackets), respectively. [b] No correlation (P < 95 %). Other r coefficients indicate the correlation significant with the confidence limit P > 99 %. [c] Ozerinina et al., 1988. [d] Zhmyrko et al., 1984. [e] Mamedov et al., 1984. [f] Zhmyrko et. al., 1987.

In Table 3 (column 1), the major positional species of TAGs are arranged in order of decreasing equivalent lipophilicity and saturated acid content (Vereshchagin, 1981). To simplify the characterization of a given geographic form as regards positional-species composition of its TAGs and the comparison of various forms with each other in this respect, we took into account only the "major" TAG species, with a concentration in a TAG mixture of at least one of the geographic forms of $\geq$ 1.0 mol %. It can be seen that the forms studied here are generally similar to each other in the number of such species.

The data of Table 3 (columns 2, 4, 6, and 8) enable one to determine the extent of difference between various forms in the positional-species TAG composition found experimentally; the results obtained are shown in Table 2 (values inside of brackets). It is seen that Group 1 forms are generally close to each other in this respect. At the same time, there is a highly significant difference between Group 1 forms and the Caucasian form in their positional-species TAG composition.

In order to understand a possible mechanism of TAG biosynthesis in the mesocarp, it was necessary to compare the quantitative composition of TAGs found experimentally with that calculated according to the existing theories of TAG structure, which were developed on the basis of various concepts of TAG formation pathways in plants (Litchfield, 1972; Vereshchagin, 1972). Previously, it has been shown that the composition of Siberian sea buckthorn TAGs, containing 32.4 mol % of saturated acids, can be accurately established without recourse to lipase hydrolysis by using only $[A]_{1,2,3}$ values and the equations of Litchfield mentioned above (Ozerinina et al., 1988; Vereshchagin, 1972). Because the saturated acid level in Central Asian and Baltic forms is similar to that in the Siberian one, we considered it possible to use the same equations for calculating the composition of their TAGs. Therefore, the values of concentrations of separate TAG positional species were calculated from the $[A]_{2calc.}$ and $[A]_{1,2,3}$ data (Table 1 and Table 1 in Ozerinina et al., 1988) by using Litchfield's equations. These values are shown in Table 3 (columns 3, 5, and 7). It can be seen that in the plants of Siberian, Baltic, and Central Asian forms they agree very closely with those found experimentally, the respective correlations being significant with P > 99 % confidence limit (r = 0.995, 0.993, and 0.947).

Because the theory of 1,3-Random, 2-Random Distribution of FAs is unsuitable for computing the TAG composition of the Caucasian form (see above), we have applied for this purpose the theory of Restricted Random Distribution of Kartha, which has been employed successfully in the past for calculating the molecular species composition of TAG mixtures that include more than 30–35 % of saturated acids (Vereshchagin, 1972). For these calculations, we used the values of $[A]_{1,2,3}$, $[S]_{1,2,3}$, and $[U]_{1,2,3}$ from Table 1, as well as those of S_3, S_2U, SU_2, and U_3 content of TAGs found according to Kartha (4.9, 43.3, 39.7, and 12.1 mol %, respectively) and the equations published earlier (Vereshchagin, 1972). The results thus obtained (Table 3, column 9) were compared with the TAG molecular species composition values found experimentally; where necessary, these values were derived by adding the concentrations of TAG positional species identical in their FA composition (Table 3, col-

Table 3

Composition of major fruit mesocarp triacylglycerol positional species of various geographic forms of sea buckthorn (mol %).[a]

Major triacylglycerol species	Geographic forms							
	Siberian [b] (*n* = 24)		Central Asian (*n* = 15)		Baltic (*n* = 21)		Caucasian (*n* = 14)	
1	2	3	4	5	6	7	8	9
PPP	0.4	0.4	0.0	0.0	0.5	0.5	4.9	4.9
POP	1.8	1.4	5.0	3.6	8.0	6.1	27.3	28.6[c]
PPO	0.1	0.1	0.0	0.2	0.3	0.3	3.0	
POO	0.2	0.2	1.4	1.3	4.6	4.6	16.7	17.3
OOO	0.0	0.0	0.1	0.1	0.7	0.8	2.6	3.5
PHP	16.2	17.3	20.9	21.2	12.2	14.8	8.1	13.4[c]
PPH	0.7	0.8	0.0	0.8	0.7	0.6	2.3	
POH	3.5	2.4	8.2	5.8	12.3	8.1	12.6	16.2[c]
PHO	2.1	2.9	5.7	7.8	7.0	11.0	5.0	
PLP	3.0	2.2	0.0	0.0	1.8	1.4	1.4	1.3
PLO	0.4	0.4	0.0	0.0	1.0	1.0	0.8	1.6
HOO	0.2	0.2	1.1	1.1	3.5	3.0	3.9	4.8[c]
OHO	0.1	0.1	0.4	0.7	1.0	2.0	0.8	
PHH	30.6	30.9	34.4	34.0	18.9	19.4	3.7	3.8
OHH	1.9	2.6	4.7	6.3	5.4	7.2	1.1	2.4[c]
HOH	1.6	1.1	3.4	2.3	4.7	2.7	1.5	
PLH	5.6	3.9	0.0	0.0	2.7	1.8	0.6	0.7[c]
PHL	1.4	2.7	0.0	0.0	0.7	1.3	0.2	
HHH	14.4	13.8	14.2	13.7	7.3	6.4	0.4	0.4
HLH	2.7	1.7	0.0	0.0	1.1	0.6	0.1	0.1[c]
HHL	1.3	2.4	0.0	0.0	0.6	0.9	0.0	
Total	86.1	85.3	99.0	97.1	91.5	90.8	94.1	99.0

[a] Columns 2 ¾ 8: positional-species composition found (even columns) from $[A]_2$ and $[A]_{1,3}$ values (Table 1) and calculated (odd columns) from $[A]_{2calc.}$ values (Table 1); column 9: molecular species composition of Caucasian form TAGs calculated from the S_3, S_2U, SU_2, and U_3 content of TAGs found experimentally (see text) and the S and U concentrations in the *sn*-1,2,3 positions of TAGs (Table 1). *n* ¾ number of TAG species. [b] Ozerinina et al., 1988. TAG mixture of Siberian form also contains TAGs of hexadecadienoic acid (Hd): PHdP, PHdH, PHHd, and HHHd; their total concentration amounts to 5.8 mol %. [c] Calculated content of a TAG molecular species equals the sum of calculated concentrations of two respective TAG positional species with the same FA composition (column 1).

umn 8). One can see that the respective data of columns 8 and 9 agree with each other ($r = 0.992$).

Fatty acid composition of sea buckthorn seed triacylglycerols

Fatty oils (i.e. TAGs) are known to be present not only in the mesocarp of sea buckthorn fruits, but also in their seeds, and seed TAGs are quite different from the mesocarp ones in their FA composition (Berezhnaya et al., 1993). Earlier, it was shown that the plants of Siberian (Berezhnaya et al., 1988, 1993; Ozerinina et al., 1987), Central Asian (Zhmyrko et al., 1978, 1984, 1987), and Caucasian (Aslanov et al., 1985; Aslanov and Mamedov, 1986; Murav'ev et al., 1985) geographic forms are more similar in this respect than in the case of mesocarp. Usually, seed TAGs include the same predominant FAs, *viz.* oleic, linoleic, and linolenic. Nevertheless, it was of interest to compare seed TAGs of various geographic forms of sea buckthorn with respect to their FA composition. Such a comparison would be essential for understanding the TAG formation process in diverse tissues of the same fruit, *viz.* the water-rich mesocarp and the drying seed.

The present results (Table 4; Table 1 in Ozerinina et al., 1987) generally confirm the previous ones: there is no significant difference between the geographic forms in the seed TAG composition. Thus, with regard to the FA composition of the seed TAGs, the individual geographic forms cannot be divided into definite groups, as could be done in the case of mesocarp TAGs. Because, in this respect, the forms studied here are close to Siberian sea buckthorn, whose TAG composition has been established earlier (Ozerinina et al., 1987), the same similarity can be suggested to occur with regard to the composition of TAGs of these geographic forms. Therefore, the TAG composition of Central Asian, Caucasian, and Baltic sea buckthorn seeds were not determined in this work.

Discussion

From Table 2 it can be seen that with respect to the FA composition of its mesocarp TAGs the wild-growing Central Asian sea buckthorn from the flood-plains of the Pyandzh river studied here is almost identical to the Uzbek sea buckthorn described earlier (Zhmyrko et al., 1984), and the Caucasian sea buckthorn from the flood-plains of the Chorokhi river is similar to the M-2 form from Azerbaijan (Mamedov et al., 1984). It follows also from Table 2 that the results on positional-species composition of TAGs agree with respective data for sea buckthorn fruits from Altai (Siberian form) and Western Pamirs (Central Asian form) published earlier (Zhmyrko et al., 1987).

The data in Tables 1-3 demonstrate that Groups 1 and 2 of sea buckthorn geographic forms are really different in both FA composition and positional-species composition of mesocarp TAGs. Based on the data of Table 2 (values outside of brackets), the Caucasian form was assumed to be distinct from the other ones in the FA composition of its TAGs, and therefore it had to be recognized as a separate group. The results on the composition of TAGs themselves (Table 2, values inside of brackets) show that the division of the geographic forms into two groups was fully justified, and there is a nearly complete analogy between the respective data of FA composition and TAG positional-species composition.

As shown in Table 1, the distinction between these two groups manifests itself primarily in the increased

Table 4

Fatty acid composition of seed triacylglycerols of various geographic forms of sea buckthorn (mol %).[a]

Fatty acid molecular species	Geographic forms					
	Central Asian	Baltic	Caucasian[b]	Siberian[c]	Central Asian[d]	Caucasian[e]
Palmitic	8.6	7.1	7.9	7.2	10.6	11.6
Hexadecenoic	2.5	1.1	0.9	1.8	5.9	4.5
Stearic	2.8	2.6	3.7	2.8	1.5	5.3
Octadecenoic	24.0	21.1	16.3	18.4	26.6	15.0
Linoleic	36.2	34.9	45.2	39.0	34.1	42.4
Linolenic	25.9	33.2	25.7	30.3	21.2	21.1
[U]	88.6	90.3	88.1	89.5	87.8	83.0

[a] Correlation between Siberian (see Table 1 in Ozerinina et al., 1988) and Central Asian; Siberian and Baltic; Siberian and Caucasian; Central Asian and Baltic; Central Asian and Caucasian; Baltic and Caucasian forms as regards FA composition of their TAGs is significant at $P > 99$ % confidence limit ($r = 0.957$, 0.986, 0.970, 0.972, 0.959, and 0.941, respectively; number of fatty acid species $n = 6$). Correlation between the experimentally found FA composition of TAGs of a given form and a previously published one is also significant ($P > 99$ %), r values for Siberian, Central Asian, and Caucasian geographic forms being equal to 0.996, 0.981, and 0.989, respectively. [b] TAGs include also myristic (0.1 mol %) and *n*-pentadecanoic (0.2 mol %) acids. [c] Zham'yansan, 1978. [d] Zhmyrko et al., 1984. [e] Aslanov et al., 1985.

content of C_{18} FAs and the reduced amount of C_{16} FAs in the TAGs of Caucasian sea buckthorn. This pattern can be assumed to be brought about by an enhanced activity, in the Group 2 fruit mesocarp, of a condensing enzyme of the FA biosynthesis system, 3-ketoacyl-acyl carrier protein (ACP) synthase II (KAS II; EC 2.3.1.41), which catalyzes the extension of saturated and unsaturated C_{16} FAs by a malonyl-CoA C_2-unit to give C_{18} FAs (Golz et al., 1994; Schuch et al., 1994; Toepfer and Martini, 1994). This assumption can be supported by recent results of Ramli and Sumbanthamurthi (1997). These workers demonstrated that, in the fruits of different varieties of oil palm, there was a strong positive correlation between the activity of KAS II in their mesocarp and the amount of total unsaturated C_{18} FAs in the oil of the latter, but a negative correlation with the palmitate content. Thus, a high concentration of C_{16} FAs in the oils of the Group 1 mesocarp tissue (75 ¾ 90 %, Table 1) can be supposed to result from a decreased KAS II activity in this tissue. When such is the case, the Baltic form must be intermediate in the magnitude of this activity between the Siberian and Central Asian forms, on the one hand, and the Caucasian one, on the other (see above).

At the same time, the difference between Groups 1 and 2 shows up not only as regards the biosynthesis of FAs, but also with reference to the formation of the TAGs themselves. The data of Table 1 demonstrate that Group 2 significantly exceeds Group 1 in the concentration of palmitate in the *sn*-2 position of TAGs (11.8 % against 0.6 ¾ 2.1 %). This pattern is in contradiction with a general feature of plant TAGs that their *sn*-2 position is almost completely occupied with unsaturated FAs (Vereshchagin, 1972).

The acylation of this position in plant acylglycerols is known to be effected by a tightly bound microsomal or plastidial enzyme, acyl-CoA:*sn*-1-acylglycerol-3-phosphate acyltransferase (lysophosphatidic acid acyltransferase, LPAAT, EC 2.3.1.51), with the formation of *sn*-1,2-diacylglycerol-3-phosphate (Hares and Frentzen, 1987); the enzyme has been identified both in seeds and in mesocarps, e.g., in the mesocarp of avocado (Eccleston and Harwood, 1992). In most plants, due to the pronounced specificity and selectivity of LPAAT for unsaturated C_{18} acyl groups, saturated FAs are excluded from the *sn*-2 position of the glycerol backbone (Cao et al., 1990). However, in the Caucasian sea buckthorn mesocarp, in contrast to the Group 1 ones, there is apparently no discrimination against the incorporation of palmitate into this position (Tables 1 and 3). In support of this statement, one can mention other instances of various forms of a same plant species displaying different acyl specificity of LPAAT. For example, erucic acid is generally excluded from the *sn*-2 position of seed oil TAGs of *Brassica oleracea*; however, by screening more than 300 accessions of this species, 9 accessions with more than 30 % *sn*-2 erucic were isolated, indicating the ability of the LPAAT to insert erucic acid into the *sn*-2 position (Taylor et al., 1994).

Finally, the distinction between Groups 1 and 2 is caused by the occurrence of different FA distribution patterns in the mesocarp TAGs of these groups. In turn, this fact is due to the functioning of different systems of TAG formation in Group 1 and 2 mesocarp. Thus, in the mesocarp of Group 1 forms, TAG biosynthesis involves random esterification of the bulk of unsaturated FAs in the *sn*-2 position; at the same time, all saturated FAs and also unsaturated ones left unesterified are distributed randomly between the *sn*-1,3 positions of glycerol moiety. As a result, the 1,3-Random, 2-Random Distribution pattern of FA residues is established (Vereshchagin, 1972).

Evidently, this pattern excludes the formation of trisaturated TAGs. On the contrary, according to Kartha's theory of Restricted Random Distribution, the occurrence of solid trisaturated TAG components (S_3) is possible, but their concentration is restricted in order to maintain the oil, being formed by a growing plant cell, in liquid state at an environmental temperature. This is exactly the case in the Caucasian form fruit mesocarp: the amount of solid S_3 glycerides actually formed (4.9 mol %) is considerably less than their theoretically possible level (11 mol %) calculated by using the 1,2,3-Random Distribution theory (Vereshchagin, 1972). Kartha's theory is based on the assumption that, after termination of S_3 synthesis, saturated acids remained unesterified, as well as total unsaturated acids, become randomly distributed between glycerol molecules, forming, however, only S_2U, SU_2, and U_3 types of TAGs. The restriction of S_3 content is an adaptive feature developed during the evolution of plants (Litchfield, 1972). Thus, we can conclude that the mesocarp TAGs of Caucasian sea buckthorn are formed according to the theory of Restricted Random Distribution of FA residues.

To the best of our knowledge, the eventual presence of two different mechanisms of assembly of an ordered structure of TAG molecules in the fruit mesocarp is demonstrated here for the first time. Meanwhile, specific biochemical mechanisms bringing about a particular pattern of FA distribution between individual hydroxy groups of the glycerol moiety in sea buckthorn mesocarp are yet to be established. For the moment, this statement holds not only for *H.rhamnoides* TAGs, but also for TAGs of all other plant species studied so far.

In conclusion, it should be emphasized that the different mechanisms of TAG formation occur only in the mesocarp, which is known to possess an exclusively maternal genotype (Berezhnaya et al., 1993). On the contrary, in seed embryo, which includes equal shares of genes of both parents, there is only one system of TAG biosynthesis, regardless of the geographic origin of plants. Thus, if it will be shown that the difference between various sea buckthorn forms in their mesocarp TAG composition is indeed caused by genotype factors, one will have to look for them only in female sea buckthorn plants.

მცენარეთა ბიოქიმია

ქაცვის გეოგრაფიული წარმოშობის გავლენა მათი ნაყოფების ტრიაცილგლიცერიდების შემცველობაზე

ა. ვერეშჩაგინი, ვ. ციდენდამბაევი

ტიმირიაზევის სახ. მცენარეთა ფიზიოლოგიის ინსტიტუტის ლიპიდების მეტაბოლიზმის ლაბორატორია, რუსეთის მეცნიერებათა აკადემია, მოსკოვი

(წარმოდგენილია აკადემიკოს თ. ბერიძის მიერ)

დახასიათებულია ქაცვის (*Hippophae rhamnoides* L.) ცალკეული გეოგრაფიული ფორმების ნაყოფის მეზოკარპის ზეთი, როგორც მათი ტრიაცილგლიცერიდების (ტაგ) შემცველობის მიხედვით, ასევე ტაგ-ის ბიოსინთეზური გზის თავისებურებების თვალსაზრისით. ციმბირის, ცენტრალური აზიისა და ბალტიის ფორმები ძირითადად შეიცავენ ჰექსადეკენოიდისა და პალმიტინის ცხიმოვანი მჟავების ნაშთებს და სინთეზირდებიან 1,3–თანაბარი, 2–თანაბარი განაწილების თეორიის მიხედვით, გლიცერინის შესატყვის ჰიდროქსილის ჯგუფებს შორის. ამავე დროს, კავკასიური ფორმების წარმოქმნა, რომლებიც მდიდარია $C_{16:0}$ და ოქტადეკენოიდის მჟავით ($C_{18:1}$), შეესატყვისება ცხიმოვანი მჟავების შეზღუდული განაწილების თეორიას. პირველადაა ნაჩვენები მეზოკარპში ტაგ-ის ბიოსინთეზის ორი განსხვავებული გზის არსებობა ერთსადაიმავე ობიექტში. ისევე როგორც თესლების ზეთის შემთხვევაში, შესწავლილი გეოგრაფიული ფორმები ფაქტობრივად არ განსხვავდებიან ერთმანეთისაგან მათი ტაგ-ის ცხიმოვანი მჟავების შემცველობის მიხედვით.

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