ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2016 Vol. 26. No. 5. 519~530 DOI : http://dx.doi.org/10.5352/JLS.2016.26.5.519

The Role of Plant Fatty Acids in Regulation of the Adaptation of Organisms to the Cold Climate in Cryolithic Zone of Yakutia

Klim Alekseevich Petrov[1,2*], Lyubov Vissarionovna Dudareva[3], Vasilii Vasilevich Nokhsorov[2], Aleksandr Aleksandrovich Perk[1], Valentin Azotovich Chepalov[1], Valentina Egorovna Sophronova[1], Victor Kirillovich Voinikov[3], Ismayil S. Zulfugarov[2,4,5] and Choon-Hwan Lee[4*]

[1]*Institute for Biological Problems of Cryolithozone, Siberian Branch, Russian Academy of Sciences (IBPC SB RAS), 41 Lenin Ave., Yakutsk 677000, Russia*
[2]*North-Eastern Federal University named after M.K. Ammosov, Institute of Natural Sciences (NEFU, INS), 48 Kulakovskogo Str., Yakutsk 677000, Russia*
[3]*Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch, Russian Academy of Sciences (SIPPB SB RAS). 132 Lermontov Str., Irkutsk 664033, Russia*
[4]*Department of Integrated Biological Science, Department of Molecular Biology, Pusan National University, Busan 609-735, Korea*
[5]*Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, Matbuat Avenue 2a, Baku AZ 1073, Azerbaijan*

Received February 5, 2016 / Revised April 29, 2016 / Accepted April 29, 2016

Vegetative plants in Yakutia are naturally frozen when they are covered with snow in the fall, and they function as green cryo-fodder that is a source of biologically active substances and nutrients for herbivorous animals. We observed a considerable increase in the total fatty acid content in the leaves of *Avena sativa*, *Elytrigia repens*, *Equisetum variegatum* and *Equisetum scirpoides* during the fall period. However, the degree of unsaturation of fatty acids was not higher in the frozen plants covered with snow than in the summer plants, with the exception of *E. scirpoides*, a dwarf horsetail found in the Pole of Cold in the northern hemisphere. In the internal adipose tissue of the Yakut horse (young horse meat), 18 fatty acids were found, including 10 saturated ones. Monounsaturated oleic C18:1 (n-9) acid and polyunsaturated α-linolenic C18:3 (n-3) acid were equally prevalent among the unsaturated fatty acids, accounting for 70% of the total unsaturated fatty acids. This composition of polyenoic fatty acids in the internal adipose tissue indicates that the Yakut horse actively feeds on the fall vegetation and the wintergreen sedge-grass. We believe that the high plant-specific free fatty acid content in the tissue of Yakut horses may play an important role in the regulation of their resistance to long-term low-temperature stress.

Key words : Adaptation, cryoresistance, fatty acids, grass plant, Yakut horse

Introduction

In the territory of Central and North-East Yakutia (the Siberian region), plants and animals face with the influence of virtually extreme cold conditions. Long-term winter frosts of high degree with temperature ranged from -40 up to -45℃ (with air temperature absolute minimum up to -68℃) and a small amount of snowpack caused by the arid harsh continental climate formed the eternal frost zone (cryolithic zone) here.

Plant developed various adaptive and defensive mechanisms to survive under adverse growth conditions [7]. In such conditions, in particular during cold hardening the local plants acquired the ability to accumulate large amounts of various substances - proteins, carbohydrates, lipids (fatty oils), carotenoids, vitamins and many other products of secondary metabolism. This ability is largely used by people and animals for nutritional purposes and for the support of a considerable part of their energy demands; it serves a source of valuable fatty acids (FAs) as well.

Since the time when the hypothesis about the role of membrane lipids in adaptation appeared [24, 31], the extensive experimental evidences concerning the influence of external conditions on their structure and composition has been collected [14, 21]. Under stress conditions, the regulation of membrane functionally active labile state is proved

***Corresponding authors**
Tel : +82-51-510-2280, Fax : +82-51-513-9258
E-mail : chlee@pusan.ac.kr (Choon-Hwan Lee)
kap_75@bk.ru (Klim Alekseevich Petrov)

to depend on the ratio of the phospho- and galacto-lipids [5, 39].

In cell membranes, mainly the FAs of the lipid molecules regulate its adaptation to the low-temperature stress [32, 37, 40]. This happens thanks to the activity of special enzymes called desaturases which is involved in classical mechanisms of hypothermia resistance [23, 27]. Membranes are the critical target at low temperatures. Cold acclimation results in their stabilization, which prevents cells death. Membrane fluidity depends directly on the changes in temperature. Changes in membrane lipid composition caused by low-temperature stress causing increase in FA unsaturation are most frequently observed. These changes allow maintaining the membrane fluidity and are essential for the survival of plants under low temperature stress [23, 26].

The main influence of low temperatures on cell membrane lipids is connected with an increase in the content of linoleic and α-linolenic acids and a decrease in the content of palmitic acid. Linoleic and α-linolenic acids are unsaturated FAs with two and three double bonds, respectively. Such apparent changes in membrane lipid composition play an important role in membrane fluidity changes. The analysis of Arabidopsis wild type lines and fatty acid desaturase (FAD) mutant lines shows that the degree of FA unsaturation or double bond index (DBI) correlates well with the membrane fluidity [41]. It has been supposed that membrane fluidity, protein conformation, cytoskeleton depolymerization and metabolic reactions are the temperature-sensitive devices [34]. The FA unsaturation levels which affect the membrane fluidity in cell membranes was considered as an important component of the temperature-sensing machinery [33].

At the same time up to now the specific data about the seasonal changes in the amounts of total lipids and their FA composition in naturally frozen fall vegetative herbaceous plants of Yakutia as well as about the influence of such type of cryo-fodder on the metabolism of herbivores are very limited. More detailed studies of these issues could contribute to the development of our earlier proposed hypothesis of the formation of a plant cryoresistance to low-temperature stress and its high nutrient value in the fall and winter under conditions of cryolithic zone [29, 30]. Thus, the purpose of the present work is to study the role of fatty acids in the regulation of the adaptation of both Yakutian plants and horse to hypothermia under conditions of cryolithic zone in Yakutia.

Materials and Methods

Materials

The leaf parts of cultivated oat (*Avena sativa* L.), couch grass (*Elytrigia repens* L.), variegated horsetail (*Equisetum variegatum* Schleich. ex Web.) and dwarf horsetail (*Equisetum scirpoides* Michx.) as well as the internal fat of the Yakut domestic horse (*Equus ferus caballus* L.) which feeds on above indicated fodders have used as materials for our study.

The cultivated oat (*A. sativa)* was grown in the experimental field (2010-2012) located in the middle bottomland of the Lena river (Central-Yakut floristic region, the outskirts of the city of Yakutsk, 62° N latitude and 130° E longitude). The green parts (leaves) of the plants grown during the standard seeding time (the end of May) and the late seeding time (the middle of July) were picked up in the beginning of August (Fig. 1A) in the first case, and in the middle of October (Fig. 1B) in the second case (the latter is the time of snow cover formation). The wild coach grass was picked up in the same area, on the meadow, during the same period as for the cultivated oat. The samples of fall- and winter-green horsetail species (2010-2013) were picked up in a

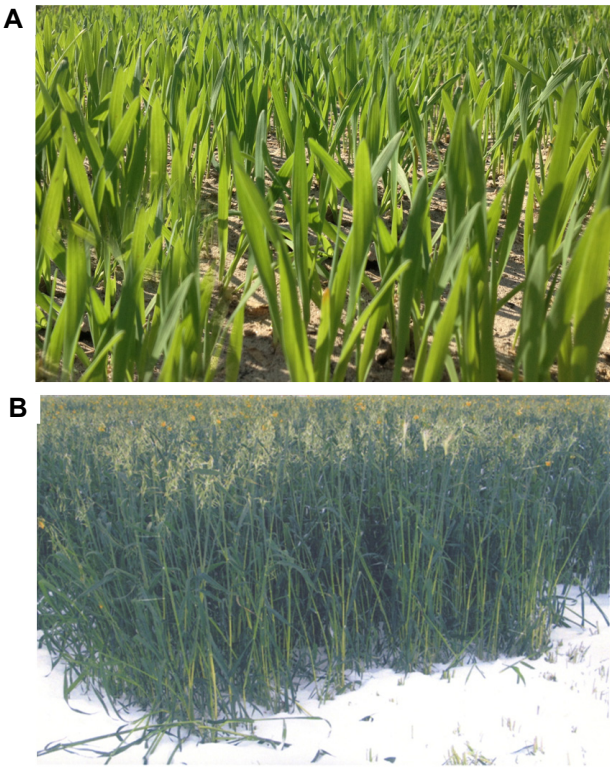

Fig. 1. The photographs of *Avena sativa* L. plants. A, taken in the beginning of August; B, in the middle of October when is the time of snow cover formation.

few river bottomlands in North-East Yakutia (Yano- Indigyr floristic region, the outskirts of the city of Verkhoyansk, the Tuostakh River middle course area, 67° N latitude and 137° E longitude) in the summer (the middle of July) and in late fall (the end of October) from under snow. Cereal leaves and horsetail cuttings (aerial parts) were fixed in liquid nitrogen and kept for further experiments after freeze-drying (VirTis, USA). The samples of the internal adipose tissue of the young domestic horse (young horse meat) were taken frozen from the killed animals in Central Yakutia in the beginning of winter (November, 2012).

Lipids extraction

Lipids extraction was carried out according to the modified method [37] with the use of the chloroform/methanol/water solvent system (1:2:0.8 v/v/v) with the antioxidant 0.001% ionol. The output of lipids was controlled using the internal standard, C19:0 acid, which was not discovered in the studied plant tissues. Rotary evaporator RVO-64 (Czech Republic) was used to remove chloroform from the lipid extract.

Methylation

To obtain the FA methyl esters (FAMEs), 1% H_2SO_4 methanol solution was added to the lipid extract after the solvent removal. The obtained mixture was heated for 30 min in a water bath at 55^0C. After cooling, FAMEs were three times extracted with hexane. The hexane extract was concentrated with the rotary evaporator, and FAMEs were cleaned by TLC method using KSK silica gel glass plates [1]. Benzene was used as a developing solvent.

GC-MS analysis

The analysis of the obtained FAMEs was carried out by a gas-liquid chromatographic method using chromatograph mass spectrometer 5973N/6890N MSD/DS Agilent Technology (USA). A highly polar capillary column HP-INNOWAX (30 m × 250 μm × 0.50 μm) was used for separation. Stationary phase was polyethylene glycol. Mobile phase was helium with gas flow velocity of 1 ml min^{-1}. Temperature of evaporator was 250°C, ion source was at 230°C, detector was at 150°C, and AUX line was at 280°C. Scanning range was 41-450 a.m.u. Mode split is 5:1. One μl of the hexane extract was injected into capillary column. The chromatographic procedure was carried out in isocratic mode at 200°C. The registration was performed by the total ion current (mode SCAN).

Identification and calculation of integral indices

FAME identification was carried out using the calculation of the equivalent length of the aliphatic chain [5]. For comparison the mass spectra library (NIST 08, Wiley 7, Christie) was used, and the retention time of the analyte was compared with that of standard compounds (Supelco®37, USA) when necessary. The abundance of FAs was determined in weight percentage of their total content among the sample under study. The content of FAMEs in the samples and the content of lipids in each fraction were determined by weighting using electronic balance GR-120 (A&N Company Ltd., Japan).

To estimate the degree of unsaturation of FAs, unsaturation coefficient (UC) and DBI was used [25]:

UC = $\sum P_{usfa} / \sum P_{sfa}$, where $\sum P_{usfa}$ is the summation of the percentage (weight %) of each unsaturated fatty acid (usfa) for all usfa, and $\sum P_{sfa}$ is that of saturated fatty acid (sfa) for all sfa;

DBI = $\sum P_j n_j / 100$, where P_j is the percentage (weight %) of j^{th} usfa and n_j is the number of double bonds in the same usfa, and DBI is the summation of $P_j n_j / 100$ for all j usfas.

The activity of the acyl-lipid ω9, ω6, and ω3 membrane desaturases responding for the introduction of double bonds into carbon chains of oleic (C18:1(n-9)), linoleic (C18:2(n-6)) and α-linolenic (C18:3(n-3)) FA was estimated by the determination of the SDR (stearoyl desaturation ratio), ODR (oleoyl desaturation ratio), and LDR (linoleoyl desaturation ratio), respectively [17], where

SDR = (%C18:1) / (%C18:0 + %C18:1);

ODR = (%C18:2 + %C18:3) / (%C18:1 + %C18:2 + %C18:3);

LDR = (%C18:3) / (%C18:2 + %C18:3).

Statistical analysis

The average data from 4 independent experiments and their standard deviations are shown in the tables. Reliability of the differences of the compared average values was estimated using t-criterion (p<0.05). The hypothesis concerning normality of the distribution was verified using Shapiro-Wilk test.

Results

We studied first the FA composition of *A. sativa* leaves

two times per year: one in the beginning of August (summer) and the other in the middle of October (fall) when is the time of snow cover formation as shown in Table 1. Total FAs were represented by 13 acids, and 8 of them were saturated and 5 of them were unsaturated.

In the FA composition, palmitic acid (C16:0) prevailed among the saturated FA; in the fall, its content increased by 20% in comparison with its amount observed in the summer. In the cultivated oat, high FA UC is composed, mainly, by α-linolenic (C18:3 (n-3)) and, to a lesser extent, linoleic (C18:2 (n-6)) acids. The contribution of the α-linolenic and linoleic acids to the oat FA balance in all seasons was 61-67% and 10-11%, respectively. Despite the fact that the value of some indices, especially of UC and SDR, decreased during the fall period, at large, a considerable increase in both total FA absolute content (2-fold) and unsaturated FA one (1,8-fold) took place in leaves. This increase causes a much higher nutritional value of late seeded and gathered plants in comparison with their summer picking.

The study of FA content in *E. repens* leaves (Table 2) gathered during the vegetation period and the period of dying-off of the aboveground organs, found 14 and 10 individual FAs, respectively. Along with the most widely-spread C16:0 palmitic acid, the following saturated FA were always present: C12:0 lauric, C14:0 myristic, C18:0 stearic, C20:0 arachic and C22:0 docosanoic acids. C16:1 palmitoleic, C18:1 (n-9) oleic, C18:2 linoleic and C18:3 linolenoic acids were discovered in all cases among the unsaturated FA like in *A. sativa*. Besides, the two latter ones were prevailing comprising all together 44% in the fall and more than 67% in the summer. At the same time, in the fall, C20:1 eicosenoic acid and the unique C18:1 (n-7) *cis*-vaccenic FA disappeared in *E. repens*. A sharp decline in unsaturated FA in *E. repens* leaves at the end of vegetation leads to the significant decrease in UC, DBI and SDR characterizing at that a substantial decrease in its feeding values. At the same time, total FA content in the fall plants, in comparison with the summer ones, was higher by 30% because of intermittent growth of saturated FA content (2.9-fold), first of all, of palmitic acid. The qualitative FA composition in *E. variegatum* appeared to be more diverse (Table 3). In the summer, 18 individual FA were identified in these species, and in the fall, there were 15 FA identified. Unlike the case with cereals, in *E. variegatum*, in addition to standard FA including the

Table 1. Total lipid fatty acid content in *Avena sativa* leaves (Central Yakutia)

Fatty acids	August		October	
	mg/g dry weight	weight content %	mg/g dry weight	weight content %
C12:0	0.35±0.01	0.41±0.69	0.90±0.03	0.54±0.27
C14:0	0.55±0.00	0.65±0.10	1.27±0.03	0.77±0.27
C15:0	0.10±0.00	0.12±0.02	0.34±0.01	0.20±0.02
C16:0	12.91±0.09	15.32±0.52	30.46±1.04	18.35±2.38
C16:1	2.43±0.02	2.88±0.11	3.34±0.08	2.01±0.03
C17:0	0.09±0.00	0.11±0.01	0.39±0.01	0.24±0.09
C18:0	1.14±0.01	1.35±0.22	5.18±0.21	3.12±1.43
C18:1(n-9)	1.43±0.01	1.70±0.11	3.39±0.04	2.04±0.25
C18:1(n-7)	0.20±0.00	0.24±0.01	0.38±0.02	0.23±0.11
C18:2(n-6)	8.53±0.07	10.12±0.15	17.99±0.25	10.84±0.75
C18:3(n-3)	56.16±0.48	66.63±1.38	101.46±2.05	61.14±1.30
C20:0	0.12±0.00	0.14±0.03	0.38±0.00	0.23±0.02
C22:0	0.28±0.00	0.33±0.02	0.48±0.01	0.29±0.02
Σ	84.29±2.68	100	165.96±3.41	100
Σ sfa	15.54±0.30	18.44±1.15	39.40±1.00	23.74±1.72
Σ usfa	68.75±1.71	81.56±1.38	126.56±2.43	76.26±1.93
UC		4.42		3.21
DBI		2.25		2.10
SDR		0.56		0.41
ODR		0.98		0.97
LDR		0.87		0.85

Values are mean ± standard error (n = 4). Σ, summation of data for all fatty acids; Σ sfa, summation of data for saturated fatty acids; Σ usfa, summation of data for unsaturated fatty acids. For the meaning of other symbols and abbreviations see Experimental section.

Table 2. Total lipid fatty acid content in *Elytrigia repens* leaves (Central Yakutia)

Fatty acids	August		October	
	mg/g dry weight	weight content %	mg/g dry weight	weight content %
C12:0	0.38±0.05	0.41±0.04	3.14±0.07	2.61±0.54
C14:0	1.44±0.03	1.57±0.18	3.68±0.06	3.06±0.33
C15:0	0.40±0.06	0.44±0.04	-	-
C16:0	17.27±1.67	18.78±0.18	47.86±0.91	39.81±6.16
C16:1	3.23±0.34	3.51±0.21	0.47±0.09	0.39±0.03
C17:0	0.27±0.09	0.30±0.07	-	-
C18:0	1.97±0.32	2.14±0.19	5.07±0.10	4.21±0.72
C18:1(n-9)	3.36±0.41	3.65±0.78	1.02±0.01	0.85±0.12
C18:1(n-7)	0.34±0.19	0.37±0.25	-	-
C18:2(n-6)	14.23±1.25	15.47±0.14	15.04±0.41	12.51±4.56
C18:3(n-3)	47.61±5.03	51.77±1.05	37.88±0.51	31.51±6.78
C20:0	0.37±0.02	0.41±0.05	3.12±0.04	2.59±0.17
C20:1(n-9)	0.07±0.04	0.07±0.04	-	-
C22:0	0.74±0.11	0.81±0.09	2.94±0.05	2.44±0.63
∑	91.69±8.17	100	120.22±0.84	100
∑ sfa	22.86±2.20	24.85±0.36	65.80±1.65	54.74±11.12
∑ usfa	68.83±5,98	74.85±0.25	54.42±0.83	45.27±11.12
UC		3.01		0.83
DBI		1.95		1.00
SDR		0.63		0.17
ODR		0.95		0.98
LDR		0.77		0.73

Values are mean ± standard error (n = 4). ∑, summation of data for all fatty acids; ∑ sfa, summation of data for saturated fatty acids; ∑ usfa, summation of data for unsaturated fatty acids. For the meaning of other symbols and abbreviations see Experimental section.

Table 3. Total lipid fatty acid content in *Equisetum variegatum* cuttings (aerial parts) (North-East Yakutia, the Pole of Cold in the Northern hemisphere)

Fatty acids	July		October	
	mg/g dry weight	weight content %	mg/g dry weight	weight content %
C12:0	0.03±0.01	0.23±0.03	-	-
C14:0	0.14±0.06	1.24±0.30	0.17±0.04	0.93±0.20
C15:0	0.04±0.02	0.36±0.11	0.09±0.01	0.49±0.04
C16:0	2.87±0.61	24.66±0.49	4.88±0.02	26.70±0.10
C16:1	0.28±0.07	2.41±0.18	0.18±0.02	0.98±0.08
C16:2(n-6)	0.12±0.02	1.02±0.06	0.13±0.01	0.71±0.05
C16:3(n-3)	0.72±0.13	6.22±0.19	0.24±0.03	1.31±0.18
C17:0	0.03±0.01	0.25±0.02	0.10±0.01	0.55±0.07
C18:0	0.25±0.07	2.13±0.14	0.60±0.05	3.28±0.27
C18:1(n-9)	0.61±0.21	5.22±0.95	1.01±0.02	5.53±0.11
C18:1(n-7)	0.06±0.02	0.54±0.07	-	-
C18:2(n-6)	1.31±0.25	11.29±0.56	4.26±0.12	23.30±0.67
C18:3(n-3)	4.60±0.88	39.51±1.08	4.62±0.04	25.27±0.19
C20:0	0.02±0.01	0.15±0.03	0.16±0.01	0.88±0.04
C20:1(n-9)	0.03±0.00	0.27±0.07	0.21±0.02	1.15±0.10
C20:3(5,11,14)	0.22±0.01	1.91±0.35	1.10±0.05	6.02±0.28
C20:4(5,11,14,17)	0.27±0.02	2.35±0.38	0.45±0.01	2.46±0.08
C22:0	0.03±0.00	0.25±0.03	-	-
∑	11.67±2.37	100	18.28±0.01	100
∑ sfa	3.42±0.78	29.27±0.86	6.14±0.11	33.59±0.55
∑ usfa	8.26±1.57	70.74±1.09	12.06±0.12	65.97±0.55
UC		2.42		1.96
DBI		1.86		1.64
SDR		0.71		0.63
ODR		0.91		0.9
LDR		0.78		0.52

Values are mean ± standard error (n = 4). ∑, summation of data for all fatty acids; ∑ sfa, summation of data for saturated fatty acids; ∑ usfa, summation of data for unsaturated fatty acids. For the meaning of other symbols and abbreviations see Experimental section.

most widely-spread saturated C16:0 palmitic and unsaturated C18:2 linoleic and C18:3 linolenoic acids, polyunsaturated ones, such as C16 – (C16:2 and C16:3) and C 20-chain length (C20-3 and C20-4) FA were observed. Frozen plants covered with snow differed much from summer-vegetating plants by the FA degree. Although the total FA content in *E. variegatum* increases almost by 60% in the fall, their quantity, at large, is not as high as it is in *Poaceae.* The most interesting phenomenon for *Equiseta* is a considerable increase in the relative content of C18:2 linoleic (2.1-fold), C20:1 eicosenoic (4.3-fold) and the unique C20:3 (5,11,14) eicosatrienoic (3.2- fold) acids as compared to the summer samples. The latter as well as C20:4 (5,11,14,17) eicosatetraenoic acids together amounted to 8.5% of all FA.

As for the other, smaller representative of fattening *Equisetum*, *E. scirpoides*, the FA contents of its summer-vegetating cuttings (aerial parts) differed pretty much from those of the fall-vegetating ones (Table 4). Fourteen FAs were identified from the summer-vegetating cuttings, and 16 FAs were identified in the fall-vegetating ones. Among the saturated FAs, palmitic acid is the most abundant in the lipid of the *E. scirpoides*. Depending on the season, its contribution amounted to 23-32% of total FA. In fall-vegetating cuttings (aerial parts) of *E. scirpoides*, new minor saturated FAs were appearing: C12:0 and C22:0. Among other saturated FA, the content of stearic C18:0 and eicosanoic C20:0 acids increased 3-fold and almost 4-fold respectively. Unlike *E. variegatum*, *E. scirpoides* does not contain *cis*-vaccenic C18:1 (n-7) and eicosenoic C20:1 acids; other unsaturated FA were met in both plants. Although in the fall, the degree of α-linolenic C18:3 (n-3) acid slightly decreased, the relative content of other unsaturated FA of C18- carbon chain and of C20- carbon chain increased: oleic C18:1 (1.9-fold), linoleic C18:2 (1.3-fold), eicosatrienoic C20:3 (5,11,14) (9-fold) and eicosatetraenoic C20:4 (5,11,14,17) acids (1.2-fold). The two latter amounted together to 5.2% of total FA in the given plant before winter determining a certain increase in UC and DBI as well in contrast to the other investigated plants (*A. sativa*, *E. repens* and *E. variegatum*). We propose that *E. scirpoides* may need additional mechanism of adaptation (FA desatura-

Table 4. Total lipid fatty acid content in *Equisetum scirpoides* cuttings (aerial parts) (North-East Yakutia, the Pole of Cold in the Northern hemisphere)

Fatty acids	July		October	
	mg/g dry weight	weight content %	mg/g dry weight	weight content %
C12:0	-	-	0.04±0.03	0.30±0.00
C14:0	0.10±0.01	0.77±0.15	0.18±0.05	1.33±0.36
C15:0	0.04±0.01	0.29±0.03	0.05±0.03	0.37±0.07
C16:0	3.94±0.04	31.60±2.57	3.14±0.04	23.26±0.17
C16:1(n-9)	0.18±0.00	1.44±0.10	0.14±0.03	1.04±0.20
C16:2(n-6)	0.15±0.03	1.20±0.19	0.11±0.02	0.81±0.15
C16:3(n-3)	0.71±0.04	5.69±0.92	0.37±0.04	2.74±0.32
C17:0	0.04±0.00	0.32±0.02	0.10±0.02	0.74±0.17
C18:0	0.23±0.02	1.84±0.27	0.74±0.61	5.48±4.41
C18:1(n-9)	0.46±0.05	3.69±0.17	0.97±0.06	7.19±0.30
C18:2(n-6)	1.64±0.28	13.11±1.41	2.28±0.08	16.89±0.57
C18:3(n-3)	4.69±0,53	37.61±1.73	4.46±0.25	33.04±1.21
C20:0	0.03±0.01	0.21±0.11	0.11±0.06	0.81±0.00
C20:3(5,11,14)	0.04±0.00	0.32±0.02	0.39±0.03	2.89±0.19
C20:4(5,11,14,17)	0.24±0.04	1.90±0.20	0.31±0.06	2.30±0.41
C22:0	-	-	0.11±0.06	0.81±0.00
∑	12.48±0.87	100	13.50±0.03	100
∑ sfa	4.37±0.09	35.04±3.10	4.47±0.62	33.11±3.78
∑ usfa	8.11±0.95	64.96±3.19	9.03±0.38	66.89±3.78
UC		1.85		2.02
DBI		1.75		1.79
SDR		0.67		0.61
ODR		0.93		0.87
LDR		0.74		0.66

Values are mean ± standard error (n = 4). ∑, summation of data for all fatty acids; ∑ sfa, summation of data for saturated fatty acids; ∑ usfa, summation of data for unsaturated fatty acids. For the meaning of other symbols and abbreviations see Experimental section.

tion) to the severe cold temperature in the North-East Yakutia that is located in the Pole of Cold in the Northern hemisphere. Thus, the leaves of *A. sativa*, *E. repens* and *E. variegatum* gain a significantly larger amount of total FA in their vegetative organs during the hardening period before winter compared with the organs in other vegetation periods.

At the same time, the presence of unique polyunsaturated FA in wintergreen horsetails is supposed to play an important role as so-called fattening of herbivores. Such fattening fodder influences the FA composition of the soft tissues of its herbivores in a certain way; therefore we have investigated the FA composition of the internal adipose tissue of Yakut horses (Table 5).

Thus, we observed an increase in total FA contents during the fall period in leaves of *A. sativa*, *E. repens*, *E. variegatum* and *E. scirpoides* (Fig. 2). However, the degree of unsaturation of fatty acids expressed as unsaturation coefficient (UC) did not increase in frozen plants covered with snow compared with summer-vegetating plants, except for the *E. scirpoides*, a dwarf horsetail living in the region of the Pole of Cold in the Northern hemisphere (Fig. 3). Among 18 FA including 10 saturated ones found in the internal adipose tissue of the horse (young horse meat), palmitic C16:0 acid, amounting to ¼ of total FA, prevailed. On the other hand, monounsaturated oleic C18:1(n-9) and polyunsaturated α-linolenic C18:3(n-3) acids were equally prevailing among the unsaturated FA amounting to 70% of their sum. Among the other polyunsaturated acids found, of special interest are eicosatetraenoic C20:4(5,11,14,17) and arachidonic C20:4(5,8, 11,14) acids. The obtained results concerning lipid FA com-

Table 5. Total lipid fatty acid content in the internal adipose tissue of the Yakut horse (winter young horse meat)

Fatty acids	mg/g dry weight	weight content %
C10:0	5.14±0.57	1.50±0.20
C12:0	16.57±0.79	4.83±0.21
C14:0	33.83±0.92	9.86±0.47
C15:0	1.38±0.11	0.40±0.02
C16:0-i	0.32±0.08	0.09±0.02
C16:0	88.36±4.53	25.75±0.68
C16:1(n-7)	32.15±1.37	9.38±0.44
C17:0-a	0.87±0.08	0.25±0.02
C16:3(n-3)	1.44±0.23	0.42±0.07
C17:0	0.60±0.07	0.18±0.01
C18:0	10.53±2.09	3.07±0.53
C18:1(n-9)	65.08±3.13	18.96±0.32
C18:2(n-6)	20.84±1.34	6.07±0.19
C18:3(n-3)	64.50±1.97	18.79±0.51
C20:0	0.14±0.09	0.04±0.01
C20:1(n-9)	0.42±0.08	0.12±0.02
C20:4(5,8,11,14)	0.72±0.34	0.21±0.09
C20:4(5,11,14,17)	0.37±0.03	0.11±0.01
Σ	343.25±11.64	100
Σ sfa	157.74±5.73	45.94±0.50
Σ usfa	185.51±6.40	54.06±0.50
UC		1.18
DBI		0.80
SDR		0.86
ODR		0.57
LDR		0.76

Values are mean ± standard error (n=4). Σ, summation of data for all fatty acids; Σ sfa, summation of data for saturated fatty acids; Σ usfa, summation of data for unsaturated fatty acids. For the meaning of other symbols and abbreviations see Experimental section.

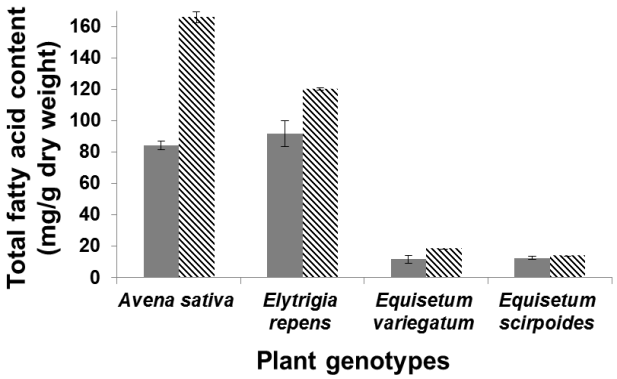

Fig. 2. Seasonal changes in total fatty acid contents in leaves of *Avena sativa*, *Elytrigia repens*, *Equisetum variegatum* and *Equisetum scirpoides*. Gray bars, Summer plants; Sketch bars, Fall plants.

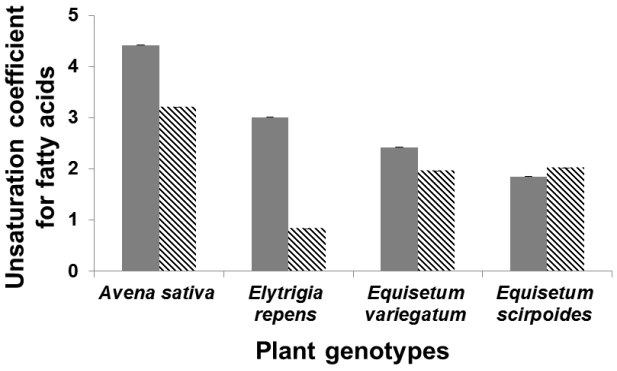

Fig. 3. Seasonal changes in the degree of unsaturation of fatty acids expressed as unsaturation coefficient (UC) of total fatty acid contents in leaves of *Avena sativa*, *Elytrigia repens*, *Equisetum variegatum* and *Equisetum scirpoides*. Gray bars, Summer plants; Sketch bars, Fall plants.

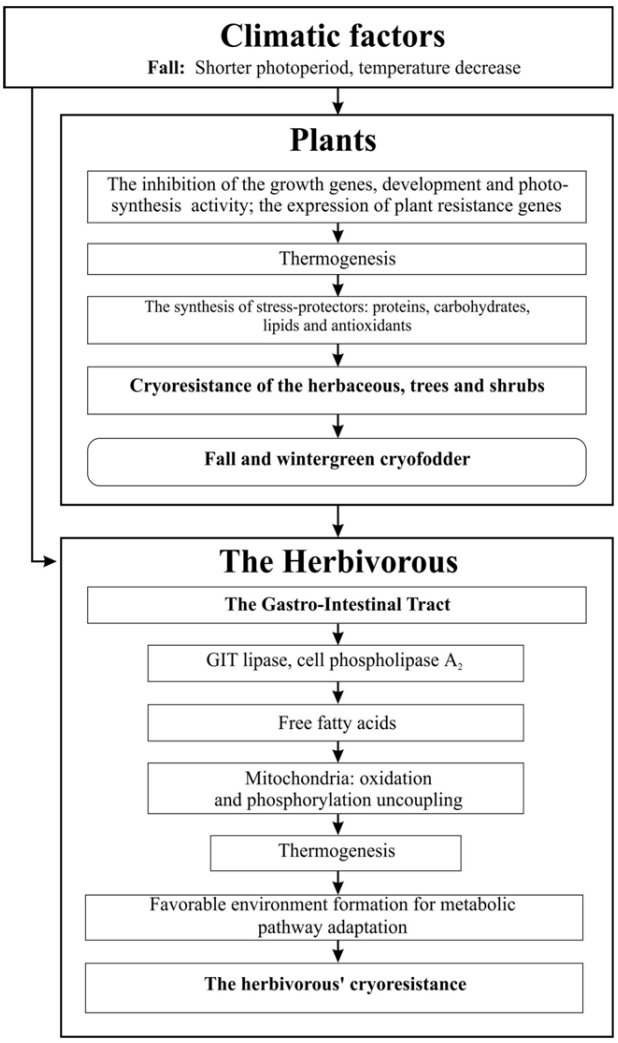

Fig. 4. The schematic model showing the role of nutrient values of fall vegetating and wintergreen plants in the adaptation and in the formation of cryoresistance of the herbivorous animals growing in the cold climate of Yakutia.

position in the adipose tissue of the Yakut horse are consistent with the data described by other authors [1, 36]. Such composition of polyenoic FA in these tissues can indicate their active income with the fodders, namely with the fall vegetating and wintergreen sedge-grass plants as well as *Equisetaceae*.

Discussion

Cold climate is an environmental factor which limits the geographical distribution and growing season of plants [8]. The peculiarities of growth, development and feed value of the herbaceous plants of Central and North-East Yakutia were of great interest for many years [3, 11, 23]. On the base of these data, we may draw a few following conclusions.

First, sedge-grasses in wet and waterlogged alas meadows, *Arctophila fulva* and cotton grass as well as horsetail plant associations of Central-Yakut and Yano-Indigyr floristic regions in Yakutia are annually subject to a long-term circumfusion with flood waters. Under these conditions, the plants keep their considerable part (up to 20-50%) in a green frozen state, and a cryopreservation of herbage as so-called cryo-fodder takes place.

Second, the cereals and sedge plants growing in alas meadows of Central and North-East Yakutia have high sustainability while being affected mechanically (pasturing and mowing of animals, hail, wind, etc.). In the affected plants, new aerial parts grow from the root-zone burgeons, but they do not have time to go through the life cycle, and the green plants are covered with snow and frozen under freezing air temperatures.

Many authors showed that both fall vegetating and wintergreen plants are distinguished by a high content of nutritional and biologically active substances which considerably prevail their quantity in the plants of other regions [2, 3, 9, 11, 30]. Taking such factors into account is important for the Northern agriculture and, especially, for the fodder production. In particular, a way of green cryo-fodder conservation covered by USSR patent No. 1835956 [35] was developed and tested for the conditions of Central Yakutia. It consists in late-summer seeding time (8-20 July) for recognized varieties of the annual cold-resistant herbaceous (*A. sativa, Brassica napus* L., *Pisum sativum* L.) which easily tolerate low temperatures (up to -7°C). The naturally-frozen plants get covered with snow in their green state, and then are gathered and used as fodder for the cattle.

During wintering, the metabolic activities of mammals are maintained by the expenditure of fat stored in the organisms during the fall period. Indeed, many mammal species living in the cold climate regions are noted for their developed ability to accumulate hypodermic, internal and brown adipose tissue (BAT) [38]. The fattening of herbivores in Yakutia takes place during a limited time. Before the hibernation, the stored fat of adult species of *Marmota sibirica* amounts to 26% of their body mass [19]. Already in mid-September, the body mass of *Spermophilus undulatus* and *Citellus parryi* as well as *Tamias* representatives increases by 20-40%; the former two species attain their maximum mass in the beginning of October, and *Tamias* representatives do a little later, usually after the animals start living in the holes [4]. The

internal fat output of *Bos taurus taurus* species amounts on the average to 8.3-8.6% of the carcass weight with subcutaneous fat thickness attaining 8-10 cm [20]. The peak fattening time of *Rangifer tarandus* species is also observed in the fall; the fat content in the rear part of the carcass amounts to 20% of the carcass weight during this period. Of great interest are the data about the fattening ability of the Yakut horse which is semi-feral during its whole life. The ability of fast fattening is one of the most remarkable features of the Yakut horse. In the fall, from August until the beginning of October, all the herds of horses already acquire their fattening when they are in good meadows; their bodies are all covered with subcutaneous fat and get rotund [11]. Various rangelands widely-spread in the territory of Yakutia – where the summer and fall vegetating herbaceous as well as wintergreen plants grow – providing food for many small and large mammals throughout the year on the natural fodder bases. During the summer and fall, the aftergrass or the grass growing in hayfields and pastures after hay-making and pasturing of cereals and sedge grass is a valuable fattening feed for many herbivores. Under favorable fall conditions the aftergrass undergoes cold hardening, remains green until the beginning of winter and gets covered with snow in the green state. Cold adaptation of fall vegetating plants to the low-temperature stress leads to the accumulation of the most energetic and antioxidant compounds, such as carbohydrates, proteins, lipids, ascorbic acid and lutein complex [30], and so in the plants in Yakutia. Plant lipids are almost insoluble in the water which makes their enzymatic splitting really difficult. This process is facilitated by fat emulsification, bile extracted by the liver being used as an emulsifier. Emulsified lipid hydrolysis begins in the duodenum under the action of an enzyme called lipase which is secreted by the pancreas and is activated under the influence of bile acids. As a result, glycerin and FAs get released, and easily passing into the bloodstream, used as the main chemical energy stored in the organism. The energy is incessantly consumed in the organism, while the food income is a periodic process. Thus, a part of the food eaten is not utilized at once, but gets stored.

During the fall/winter period, animal fats are characterized by a high content of palmitic, stearic, and oleic acids, the sum of which can exceed 3/4 of total FA included into the fat [30]. At the same time, unsaturated FA content in the fat of the aboriginal Yakut cattle is considerably higher than the one in the fat of other cattle breeds [20].

The question of accumulation of polyunsaturated FAs including linoleic and α-linolenic acids in the fat of Yakut horse is of particular interest, because these FAs are not synthesized in the body of herbivores; they are provided from the vegetative fodder. The results shown in Table 5 on lipid polyunsaturated FA composition in the adipose tissue of the aboriginal hoofed animal are consistent with the published data [1]. The following regularity is found in cervine and pork fat: the content of palmitic, stearic and oleic acids amounts are 27%, 18%, and 30%, respectively [36]. At the same time, the fats of Yakut horse are mostly represented by palmitic (from 16.2 to 21.4%), oleic (from 20.4 to 24.6%) and α-linolenic (from 12.3 to 18.3%) acids in different parts of the carcass, and their sum exceeds the half of the total FAs [36]. Our data presented in this work also confirm this fact. Thus, the polyunsaturated FA content in the fats of aboriginal Yakut horse is noticeably higher than the one in the fats of other domestic animals.

The fat composition of Yakut horse is unique among the domestic animals. The level of α-linolenic acid in the adipose tissue of the Yakut horse was proved to be considerably higher than the one in the adipose tissues of amphibians, reptiles, birds, rodents and many herbivores and carnivores [16]. The fat of pasturable horses was shown to differ noticeably from the fat of the horses fed with a dietetic food concentrate [6]. This fact demonstrates that the habitat and the peculiarities of the fodder used have a strong influence on the fat composition of animal tissues. The FA composition of the horses fed with natural pasture fodder is characterized by a low level of stearic acid and a very high content of α-linolenic acid [6]. FAs of the C18 unsaturated group, mostly linoleic and α-linolenic acids, prevail in them. The fat of Yakut horse is similar to the pork fat in its composition; it is only a high level of α-linolenic acid and a little smaller content of stearic acid in the former that makes the difference between them [10]. The published data and the results obtained in the present work show very high level of C18:3 in the fat which is closely connected with peculiarities of the pasture diet of Yakut horse.

It is common knowledge that the BAT mentioned above represents special mammalian fat with a large number of mitochondria. That is why this tissue is able to produce much heat energy in a very short time. BAT plays the role of "a spark plug" which gives the first impulse to self-heating of the organism [15, 19]. The BAT mitochondria have the unique peculiarity – their inner membrane contains an

uncoupling protein (UCP1). The thermogenic function of the BAT is mainly conditioned by activity of this protein [13, 18, 28]. UCP1 functions as a highly regulated proton transporter which does it through the inner membrane of mitochondria and gets inhibited by purine nucleotides and activated by free fatty acids (FFA) [13, 28].

Indeed, the freezing of the animals adapted to the cold causes the increase in FFA content in their tissues [22]. According to contemporary opinions, un-etherified FA in the animal energy metabolism exercises a function of double nature: on the one hand, FFA provokes energy dissipation as uncouplers of oxidative phosphorylation, on the other hand – FFA is the source of energy (the substrate for mitochondrial respiration). It is due to the increase in the quantity of the given FAs in cells, that the decrease in the association of oxidation and phosphorylation processes provoking heat generation takes place. At the decrease in temperature, there is phospholipase A_2 activation which, in its turn, results in FFAs accumulation and causes changes in the cell energy. That is why FAs in cell mitochondria do not only become the main oxidative substrates, but are also the most important regulators-uncouplers of oxidation and phosphorylation in the respiratory chain.

The schematic model showing the role of nutrient values of fall vegetating and wintergreen plants in the adaptation and in the formation of cryoresistance of the herbivorous animals growing in the cold climate of Yakutia.

The scheme below (Fig. 4) describes the dependency of cryoresistance of the herbivores on from climatic factors. Thus, the climatic factors, first of all, the reduction in the day length and the decrease in average daily temperatures, influence two consecutive hardening phases of the fall vegetative herbaceous plants. The second phase – hardening by temperatures below zero – is only possible after the influence of low positive temperatures which is the first phase. The hardening phase induces the synthesis and deposition of a large number of different energy-intensive substances in plants including lipids and FAs. The same condition, on the other hand, causes hormonal changes in the organisms of the local herbivores that are fed on green cryo-fodder; Hormonal changes help reorganization of the metabolism of the herbivores to prepare for overwintering at extremely low temperature. During the overwintering period, lipase splits triglycerides contained in the fat pools accumulated since the fall, into FAs and glycerin. The FFA formed in the mitochondria in this way acts not only as the main oxidation substrates, but also as the significant regulator – respiration and phosphorylation uncoupler facilitating the transformation of respiratory substrate energy into heat.

We have reported herein a study of the FA compositions of plants and horse and based on the results of our studies concerning the significant content of free FAs (saturated and unsaturated) in the tissues of fall vegetating and wintergreen plants covered with snow and we suppose that their fatty oils may play an important role in the regulation of the resistance of herbivores to a long-term low-temperature stress. Although this research done for Yakut horses and plants grown in Yakutia, we believe that same circumstances is true for other cold regions of the World.

Acknowledgement

The work of ISZ and CHL was supported by a 2-Year Research Grant of Pusan National University.

References

1. Abramov, A. F. and Petrova, L. V. 2010. Fatty acid content in the meat of the Yakut breed foals. *RASHN Papers* **3**, 56-57.
2. Alekseev, V. G. 1994. *Plant resistance in the North conditions: ecological and biochemical aspects*. Nauka: Novosibirsk.
3. Andreev, V. N., Galaktionova, T. F., Govorov, P. M., Zakharova, V. I., Neustoyeva, A. I., Savinov, D. D. and Torgovkina, E. E. 1978. *Seasonal and weather dynamics of phytomass in the subarctic tundra*. Nauka: Novosibirsk.
4. Anufriev, A. I. 2008. *The mechanisms of small mammals hibernation in Yakutia*. RAN: Novosibirsk.
5. Bocharov, E. A. and Djanumov, A. A. 1978. The synthesis of polar lipids in winter wheat chloroplasts due to the plant cold-resistance. *Plant Physiol. (Moscow)*. **25**, 756-760.
6. Bowland, J. P. and Newell, J. A. 1974. Fatty acid composition of shoulder fat and perinephric fat from pasture-feed horses. *Can. J. Anim. Sci.* **54**, 373-376.
7. Cui, M., Lin, Y., ZU, Y., Efferth, T., Li, D. and Tang, Z. 2015. Ethylene increases accumulation of compatible solutes and decreases oxidative stress to improve plant tolerance to water stress in *Arabidopsis*. *J. Plant Biol.* **58**, 193-201.
8. Dong, C. H. and Pei, H. 2014. Over-expression of miR397 improves plant tolerance to cold stress in *Arabidopsis thaliana*. *J. Plant Biol.* **57**, 209-217.
9. Egorov, A. D. 1960. *The chemical composition of fodder plants of Yakutia*. Academy of Sciences USSR: Moscow.
10. Elliot, J. I. and Bowland, J. P. 1968. Effects of dietary copper sulfate on the fatty acid composition of porcine depot fats. *J. Anim. Sci.* **27**, 956-960.
11. Gabyshev, M. F. 1957. *The Yakut horse*. Yakutsk book publishers: Yakutsk.

12. Gabyshev, M. F. and Kazansky, A. V. 1957. *Fodder grasses of Yakutia. Characteristics of the chemical composition and nutrient value of the fodder grasses in the Yakut ASSR*. Yakutsk book publishers: Yakutsk.
13. Garlid, K. D., Jaburek, M. and Jazek, P. 1998. The mechanism of proton transport mediated by mitochondrial uncoupling proteins. *FEBS Lett.* **438**, 10-14.
14. Harwood, J. L. 1994. Environmental factors which can alter lipid metabolism. *Prog. Lipid Res.* **33**, 193-202.
15. Heldmaier, G., Buchberger, P. and Seidi, K. 1983. Contribution adipose tissue to the regulatory heart production in the Hungarian hamsters. *J. Therm. Biol.* **41**, 305-321.
16. Hilditch, T. P. and Williams, P. N. 1964. *The chemical constitution of natural fats*. 4th ed., Chapman and Hall: London.
17. Jaworski, J. G. and Stumpf, P. K. 1974. Fat metabolism in higher plants. Properties of a soluble stearyl-acyl carrier protein desaturase from maturing *Carthamus tinctorius*. *Arch. Biochem. Biophys.* **162**, 158-165.
18. Jezek, P., Engstova, H., Zackova, M., Vercesi, A. E., Costa, A. D. T., Arruda, P. and Garlid, K. D. 1998. Fatty acid cycling mechanism and mitochondrial uncoupling proteins. *Biochim. Biophys. Acta.* **1365**, 319-327.
19. Kalabukhov, N. I. 1985. *The mammals' hibernation*. Nauka: Moscow.
20. Korotov, G. P. 1966. *The Yakut cattle: productivity and biological peculiarities*. Yakutsk book publishers: Yakutsk.
21. Kuiper, P. J. C. 1984. Lipid metabolism of higher plants as a factor in environmental adaptation. *Dev. Plant Biol.* **9**, 525-530.
22. Levachev, M. M., Mishukova, E. A., Syvkova, V. G. and Skulachev, V. P. 1965. The energy exchange of the pigeon during its self-heating after hypothermia. *Biochemistry (Moscow).* **30**, 864-871.
23. Los, D. A. and Murata, N. 2004. Membrane fluidity and its role in the perception of environmental signals. *Biochim. Biophys. Acta.* **1666**, 142-157.
24. Lyons, J. M. 1973. Chilling injury in plants. *Ann. Rev. Plant Physiol.* **24**, 445-466.
25. Lyons, J. M., Wheaton, T. A. and Pratt, H. R. 1964. Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plants. *Plant Physiol.* **39**, 262-268.
26. Mikami, K. and Murata, N. 2003. Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Prog. Lipid Res.* **42**, 527-543.
27. Nishida, I. and Murata, N. 1996. Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 541-568.
28. Palou, A., Pico, C., Bonet, M. L. and Oliver, P. 1998. The uncoupling proteins thermogenin. *Int. J. Biochem. Cell Biol.* **30**, 7-11.
29. Petrov, K. A., Sofronova, V. E., Chepalov, V. A., Perk, A. A. and Maksimov, T. Kh. 2010. Seasonal changes of photosynthetic pigments content in perennial herbaceous of cryolithic zone. *Plant Physiol. (Moscow).* **57**, 192-199.
30. Petrov, K. A., Sofronova, V. E., Buryakina, V. V., Perk, A. A., Tatarinova, T. D., Ponomarev, A. G., Chepalov, V. A., Okhlopkova, Zh. M., Vassylieva, I. V. and Maksimov, T. Kh. 2011. Woody plants of Yakutia and low-temperature stress. *Plant Physiol. (Moscow).* **439**, 866-874.
31. Raison, J. K. 1973. The influence of temperature-induced phase changes on the kinetics of respiratory and other membrane-associated enzyme systems. *J. Bioenerg.* **4**, 258-309.
32. Ramos, P., Gieseg, S. P., Shuster, B. and Esterbauer, H. 1995. Effect of temperature and phase transition on oxidation resistance of low density lipoprotein. *J. Lipid Res.* **36**, 2113-2128.
33. Routaboul, J. M., Fisher, S. F. and Browse, J. 2000. Trienoic fatty acids are required to maintain chloroplast function at low temperatures. *Plant Physiol.* **124**, 1697-1705.
34. Ruelland, V. and Zachowski, A. 2010. How plants sense temperature. *Envir. Exp. Bot.* **69**, 225-232.
35. Rumyantsev, V. A. 1990. A method of green fodder storage. USSR Patent No 1835996.
36. Slobodchikova, M. N., Ivanov, R. V., Stepanov, K. M., Pustovoy, V. F., Osipov, V. G. and Mironov, S. M. 2011. Lipid fatty acid composition of the fat tissue of the Yakut horse. *Horse Breed. Eques. Sport.* **6**, 28-30.
37. Smolenska, G. and Kuiper, P. J. C. 1977. Effect of low temperature on lipid and fatty acid composition of roots and leaves of winter rape plants. *Physiol. Plant* **41**, 29-35.
38. Solomonov, N. G. 1976. Major results and objectives of ecological and physiological research of wild mammals in Yakutia, pp. 3-24. In *Ecological-physiological peculiarities of the animals of Yakutia*. Nauka: Novosibirsk.
39. Sopyn, A. I. and Trunova, T. I. 1991. Dynamics of phospholipid and fatty acid content in etiolated winter wheat seedlings during frost hardening. *Plant Physiol. (Moscow).* **38**, 142-149.
40. Thewke, D., Kramer, M. and Sinensky, M. S. 2003. Transcriptional homeostatic control of membrane lipid composition. *Biochem. Biophys. Res. Commun.* **273**, 1-4.
41. Tovuu, A., Zulfugarov, I. S. and Lee, C. H. 2013. Correlations between the temperature dependence of chlorophyll fluorescence and the fluidity of thylakoid membranes. *Physiol. Plant.* **147**, 409-416.

초록 : 야쿠티아의 동토지역에 서식하는 생물의 추운기후-순화의 조절에서 식물 지방산의 역할

페트로프[1,2*] · 두다레바[3] · 노크소로프[2] · 퍼크[1] · 세파로프[1] · 소프로노바[1] · 보이니코프[3] · 쥴푸가로프[2,4,5] · 이춘환[4*]
([1]러시아 과학아카데미 동토생물문제연구소, [2]러시아 북동연방대학교, [3]러시아 시베리아 식물생리생화학연구소, [4]부산대학교 분자생물학과, [5]아제르바이쟌 분자생물생물공학연구소)

자연상태에서 녹색인 채로 눈에 덮여 있는 냉동사료 엽채식물은 초식동물의 생물활성물질과 영양분의 근원이 된다. 우리는 *Avena sativa*, *Elytrigia repens*, *Equisetum variegatum*과 *Equisetum scirpoides*의 잎에서 가을에 전체 지방산 함량이 상당히 증가함을 관찰하였다. 그러나, 여름에 채취한 잎에 비하여 눈에 덮혀 얼어있는 식물에서 지방산 불포화도의 증가는 없었다(북반부 온도최저점 지역에 서식하는 키가 작은 쇠뜨기인 *E. scirpoides*는 예외임). 야쿠티아 말의 내부 지방조직(젊은 말의 고기)에서, 18개의 지방산이 분석되었고, 그 중 10개가 포화 지방산이었다. 그 중 70%에 해당하는 양으로 단일불포화 올레산(C18:1(n-9))과 고도불포화 α-리놀렌산이 비슷하게 분포하였다. 내부 지방조직에 이렇게 많은 고도불포화 지방산이 많이 분포하는 것은 가을과 겨울에 먹인 녹색 먹이의 영향으로 본다. 우리는 야쿠티아 말의 조직에 상당한 양으로 분포하는 식물 특이적 지방산 함량은 이 말들이 오래 동안 저온 스트레스 상황에서 저항성을 가질 수 있은 조절기작에 중요한 역할을 한다고 제안한다.