ECO-PHYSIOLOGICAL AND BIOCHEMICAL BASES OF THE GREEN CRYO-FEED FORMING IN YAKUTIA (review)

K.A. PETROV¹, A.A. PERK¹, V.A. CHEPALOV¹, V.E. SOFRONOVA¹, A.N. ILIYN², R.V. IVANOVA

¹Institute for Biological Problems of Cryolithozone Siberian Branch of RAS, Federal Agency of Scientific Organizations, 41, pr. Lenina, Yakutsk, Sakha Republic (Yakutia), 677000 Russia, e-mail kap_75@bk.ru (corresponding author K.A. Petrov);
²M.G. Sofrononov Yakutsk Research Institute of Agriculture, Federal Agency of Scientific Organizations, 23/1, ul. Bestuzheva-Marlinskogo, Yakutsk, Sakha Republic (Yakutia), 677001 Russia

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Food grown in Yakutia can be used as a supplement to the diet of many farm animals in Siberia, the Far East and the European North. Understanding mechanisms of cold adaptation in plants, being of general biological significance, also makes basis for use of this practically unlimited natural forage resource. Here, we summarized ecological, physiological and biochemical aspects for the formation of nutritional value of autumn-vegetating herbaceous plants which were frozen with natural cold have been considered. These plants are highly nutritious, autumn/winter fattening food (green cryo-feed), for herbivores in the extremely harsh conditions of the North. In the article an overview data on plant response to low temperatures and their adaptation is provided (T.I. Trunova, 2007; L.V. Gusta et al., 2013; K. Miura et al., 2013). As a result of generalization of long-term authors’ investigations (A.Ya. Perk et al., 1987; K.A. Petrov et al., 2010; A.N. Ilyin et al., 2015) and other publications, the general theory is developed of the mechanisms of resistance of plants and animals to prolonged hypothermia in the conditions of permafrost (cryolithozone) in Yakutia. It is assumed that adaptation of plants to long-term low temperature stress have closely connected with their main source of energy (lipids, unsaturated fatty acids), which plays a major role in the formation of high nutritional value of autumn-vegetating plants which were frozen with natural cold in the permafrost zone. Cold hardening of perennial herbaceous plants in the cryolithozone of Yakutia is evidently due to cell accumulation of primary and secondary carotenoids with the most pronounced antioxidant properties (B. Demmig-Adams et al., 2006). Green cryo-feed provides vital activity of herbivores, including livestock (the Yakut horse, reindeer, etc.), under conditions of prolonged and extremely cold winter. Green cryocorn technology allows to provide animals with protein, fatty oils, carbohydrates and vitamins throughout the whole wintering period. As a result of our experiments have been shown highly nutritional value of green cryo-feed for feeding of livestock, for example for herd horse breeding, that allows to recommend the cryo-feed for wide introduction into agricultural practices in regions where unlimited resources of cold could be used.

Keywords: Yakutia, adaptation to cold, green cryo-feed, nutritional value

Cold resistance means ability of the plants to resist low positive temperatures, frost tolerance (frost resistance) means tolerance to temperatures below zero during winter. Winter resistance, including frost resistance, means complex resistance to unfavorable factors of winter season, namely frost, thaws, ice crust, asphyxiation, heaving, rotting, etc. [1-4]. In the past decades, frost resistance was considered as ability of the plants to prevent formation of ice inside cells and to enhance resistance to intracellular ice under the effect of negative temperatures [5-7]. Chill resistance (ability to resist relatively short-term effect of...
negative temperatures) is also important [2].

The Republic of Sakha (Yakutia) is among the coldest regions of the world. Winter temperatures may fall below –60 °C. Nevertheless, Yakut horses and other herbivorous animals survive here at open air all year around. Evolution origins of Yakut horses and genetic basis of their adaptation still remain unresolved [8], most probably these horses have appeared after migration of the Yakut people few centuries ago, and represent one of the most vivid examples of fast adaptation to extreme conditions of criolithozone. In our view, high-energy biologically active substances accumulated in organs of green cryo-feed in the autumn-winter period play the key role in regulation of adaptation to the extreme conditions of criolithozone. Understanding mechanisms of cold adaptation in plants, being of general biological significance, also makes basis for use of this practically unlimited natural forage resource. Food grown in Yakutia can be used as a supplement to the diet of many farm animals in Siberia, the Far East and the European North. It should be noted that Yakut breed of horses is unique by high concentration of essential fatty acids in its meat playing an important role in human metabolism [9-11].

In this review we have considered environmental, physiological, and biochemical aspects for formation of the nutritional value in naturally frozen autumn-vegetative Northern plants which serve highly-nutritional autumn-winter fattening feed (green cryo-feed) for herbivores in extremely harsh conditions of their habitation.

Reaction of plants to low temperature exposure. Plant winter resistance has been investigated for a long time with frequent discussion of the reasons of destruction from frost (loss of heat, vessel rupture, dehydration, formation of ice, acidification and strengthening of cell fluid, attainment of the specific temperature minimum, and etc.). Research results of the physiological aspects of plant adaptation to low temperatures are provided in more detail in a series of study overviews [6, 11-18].

There are three types of cell death in freezing [5], i.e. due to rapid formation of intracellular ice initially focused in cytoplasm and further in vacuole; due to dehydration and deformation resulted from intracellular ice formation; due to formation of inter- and intra-cellular ice. “Winterkill” type depends of the physiological state of plants, of their readiness for wintering. Damage of plants upon formation of ice, mainly intracellular ice, results in dehydration and mechanical deformation of cytoplasm, and finally in structural rupture of protein and lipid components of membranes due to enzymatic hydrolysis of phospholipids and due to formation of phosphoric acid. The root cause of damage and loss of cold-resistant and heat-loving plants due to low temperature exposure is transformation of liquid crystal membranous lipids into a solid gel. Phase change in lipids results in the increased size of membranous pores, which is both accompanied by their semi-permeability loss and inactivation of sugar and K⁺ ion active transport systems, as well as by faster water discharge from cells and, thus, causing killing of a plant [19].

Adaptation of plants to low temperature stress. Formation of physiological mechanism of resistance to unfavorable wintering conditions involves rest and hardening stages [20]. This mechanism prevents formation of ice inside a cell under the effect of negative temperatures and ensures higher resistance to intercellular ice that reduces dehydration and mechanical deformation of protoplast. Cryo-resistance is realized at various levels, from organismic to molecular, provided that cryo-resistance mechanisms in woody and herbaceous species have certain characteristics. Thus, at the end of vegetation period as a day becomes longer all buds of woody species at mid and high latitudes
go into the state of deep physiological rest that allows them overcoming the unfavorable period. As apart from woody species, herbaceous species do not go into the state of physiological rest, but their growth is suspended under the effect of low temperatures (0-5 °C). Not only low positive temperatures, but also the light is required for hardening. At that, hardening ability is different in winter cereals at various ontogenesis stages: the closer is generative stage, the lower is the hardening ability [21, 22].

Cold hardening of herbaceous plants significantly changes metabolism, whereby I.I. Tumanov [20] had distinguished two consecutive hardening phases. To this end, it occurs only in case when first negative temperatures (second phase) effect after low positive ones (first phase). Cryo-protector formation process is mostly expressed during the first hardening phase. Cryo-protectors are low- and high-molecular compounds: carbohydrates, lipids, proteins, etc. Due to cryo-protectors a cell gets more protection against formation of intracellular ice and dehydration. The most intensive increase in sugars and lipids, main nutritional substrates of respiration in plants, is noted. Increase of sugars promotes water retention in a cell in unfrozen state, which prevents denaturation of proteins [6]. Besides, cold hardening significantly increases concentration of lipids, phospholipids, and unsaturated fatty acids that provided for decrease of temperature of transformation of membranous lipids from a liquid crystalline state into the gel-like state [23-26]. Such temperature is lower than freeze point in frost resistant plants, and is above 0 °C in nonresistant plants.

Free fatty acids (FFA), not included in membranous lipids, may influence on the activity of mitochondria. Transfer of mitochondria into a low-energy state in frost-resistant winter cereals was noted about 30 years ago by V.K. Voynikov at al. (27, 28). Upon such transfer, FFA content in mitochondria had increased 2-3 times, and within a half an hour the seedlings had retained the temperature which was 7-10 °C higher than air temperature. All such effects were not found in summer cereals. Upon hypothermia and in control, FFAs were mainly presented by C_{16}-C_{20}: accounting for 84 % of the total fraction, whereat unsaturated acids comprised the most part of the total amount of fatty acids amongst C_{16}-C_{20}. Phospholipase A_2 activates upon reduction of temperature, thus, resulting in accumulation of FFA and changes in the nutritional processes in a cell. Fatty acids in mitochondria become not only the main oxidation substrates but also the most important regulator, i.e. the disconnector of respiration and phosphorylation, facilitating transformation of energy of respiratory substrates into the heat upon hypothermia [29, 30]. First of all, it is noted by activation of the alternative oxidase, plant uncoupling mitochondrial protein PUMP and stress protein CSP 310 causing thermogenesis and local temperature increase. It allows plants to gain time for adaptation change of metabolism, e.g. change of the content and structure of membranes, transportation of required metabolites through membranes, synthesis of cell dehydration stress proteins (anti-freeze, chaperons, dehydrins and disconnectors) and etc. [27, 28, 31, 32].

The second hardening phase in herbaceous plants is characterized by significant structural cell changes. Intercellular ice is formed in autumn vegetative species under the effect of negative temperatures, which prevents formation of ice within cells. In this period, there are two main adaptation mechanisms: stronger discharge of free water from plant cells through the membranes and protection of cell components from dehydration consequences. Water is discharged through membranes due to an increase in concentration of unsaturated fatty acids [6, 33]. Changes in phospholipids also affect the membrane features and increase its permeability for water, whereat rapid reduction of phospholipids upon freezing results in stronger water discharge in intercells and protects a cell
from intracellular ice formation [34].

Green cryo-feed. In mid latitudes air temperature often falls till −20...−40 °C. Permafrost zone lays to the north, where air temperature is even lower. The main point of seasonal vegetation growth and development dynamics in the cryolithozone is that termination of growth processes and going of perennial plants into the state of deep physiological rest with simultaneous sharp reduction of photosynthetic activity coincides with the second part of August — beginning of September and coincides with the period of maximum soil respiration intensity and the most formation of seasonally-thawed stratum. After-grass and many autumn-vegetative herbaceous species, which are subjected to hardening, remain green till late autumn and in such state move under the snow. It should be noted that in cryolithozone of Yakutia, as well as in other Siberian regions with similar climate, such unfavorable events as rotting, asphyxiation and heaving due to the lack of recursive warming widely occurring in the regions with soft climate are kept to a minimum at beginning of winter season.

Main and specific aspects of climate in Central and North-Eastern Yakutia is extra-continental conditions with low amount of precipitations (200-250 mm), strongly shortened (up to 80-120 days) frost-free period due to late and early chill, significant temperature fluctuations within a year (up to −60 °C in winter and up to +40 °C in summer), unusual dryness of cold air, as well as availability of eternal frost. Thus, green cryo-feed — wintergreen herbaceous species, which serve winter-grazing food for most animals — is widely used in Yakutia [35, 36].

Study of growth and development characteristics [37], as well as nutritional value of herbaceous plants in Yakutia [38-41], had shown that here cereal-sedge plant formation is often subjected to mechanical damages (pasturing, domestic mowing, etc.) and long-term flooding. Vegetation of damaged and underwater plants starts later, they often can’t go fast enough the entire growth and development cycle, and, going under the snow, preserve significant part in a green frozen state, in which case, green mass passes cryo-preservation in a form of fattening feed [41].

Nutritional value of green cryo-feed. Study of the extensive material had shown that during winter wintergreen parts of wild herbaceous plants always preserve high concentration of nutritional and biologically active substances [39-42], which is important for Northern agriculture, especially for feed production. First zoo technical studies confirming the effectiveness of use of the green plants of late and summer-time sowing, being preserved with the use of the natural cold, have been conducted in Khakassia in 1947 at Krasnoyarsk experimental station [43, 44]. In furtherance, patented green cryo-feed making method was developed and tested in conditions of the Central Yakutia [45, 46]. It involves late summer sowing terms (July 8-20) of the released variety of anotinous cold-resistant herbaceous plants — oat (Avena sativa L.), rape (Brassica napus L.), pea (Pisum sativum L.) that perform well under the effect of temperatures of up to −7 °C. Naturally frozen plants go under snow in green state. Afterwards, they are gathered and used as cattle feed.

Autumn in Yakutia is characterized by the most favorable weather conditions for improvement of thermal resistance of autumn-vegetative herbaceous plants. Predominant meteorological elements involve lots of bright sunshine days required for photosynthesis and chilly nights allowing suspending the consumption of carbohydrates for respiration. According to long-term average data, period in the Central Yakutia with temperatures suitable for passing of the first hardening phase (daily temperatures reaching +10...+15 °C, and nighttime temperatures reaching −1...−2 °C) coincides with the II–V pentads of September [47,
It is the period when ability of plants to overcome first negative temperatures due to cold hardening is gradually formed.

We have conducted comprehensive ecophysiological and biochemical studies for cold-resistance in the released local-breed annual and perennial Yakut species of herbaceous plants: common oat (A. sativa L.) Nyurbinsky variety and awnless brome (Bromopsis inermis Leys) Ammachaan variety. Cereals planted in conditions of the Central Yakutia were subjected to natural cold hardening during the second half of September. In pursuance thereof, awnless brome was sown during the period optimal for the climate region (end of May — beginning of June), afterwards, during the heading commencement phase (II decade of July), plants were cut to promote lying of new vegetative shoots. Alternatively, common oat was sown in later periods (in the mid of July) significantly shifted as compared to commonly accepted terms (end of May — beginning of June). Growing shoots of awnless brome and common oat were subjected to natural hardening by low positive temperatures (from 0 to +5 °С). At beginning of October, naturally frozen plants went under the snow during the stem elongation phase. These studies allowed examination of effect of the first and second cold adaptation phases on herbaceous plants of Yakut cryolithozone.

In high latitudes, adaptation of winter-resistant plants to low temperature is due to adaptation of photosynthetic apparatus (PSA), i.e. shift of assimilation optimum CO₂ to lower temperatures with an increase in CO₂ assimilation rate [49]. Hardening cells of frost-resistant plants are rich in photosynthesis products since photosynthetic intensity in such plants (as apart from non-resistant species) at near-zero temperatures significantly exceeds the respiratory activity [49-53]. It results in accumulation of the large amount of sugars playing multifunctional role at low-temperature adaptation in plants [6].

Field studies had shown significant increase of carotenoids of violaxatine cycle in all studied species of perennial cereals in the Central Yakutia at beginning of autumn chilling simultaneously with growth of the amount of chlorophyll. The same pattern was observed in horsetails in the cold pole region, provided that here quantity of carotenoids had increased 1.2-3.0 times as compared to summer values upon simultaneous increase of the content of oxygen-containing substances and reduction of the amount of chlorophylls compared to xanthophylls and carotenoids. In general, vegetables were characterized by low content of chlorophylls and carotenoids which is due not only to the extremely severe conditions in the region, but also to specificity of plants of Equisetaceae family. Close to winter, an increase in the amount of secondary carotenoid, rhodoxanthin, was observed in the studied horsetail species. Probably, in such circumstances, rhodoxanthin plays the more important antioxidant role than violaxatine cycle, which is unable to operate at constant low temperatures [39].

Accordingly, one of properties of the cold hardening of perennial herbaceous plants in the cryolithozone of Yakutia is clearly high concentration in the cells of primary and secondary carotenoids with most expressed antioxidant properties. Carotenoids protect light harvesting complex of PSA from excitation energy surplus at high intensity of light [54] which, in conditions of autumn low positive temperatures, allows plants to timely complete the first phase of cold hardening. The later results in accumulation of the large amounts of high-energy compounds, important plastic substrates and bioactive substances such as sugars, proteins, fatty acids of bulk lipids and antioxidants (polyenoic fatty acids, vitamin C, β-carotene, lutein complex, etc.) [6, 39-41], which significantly improve nutritional value of autumn-vegetative and wintergreen herbaceous plants in cryolithozone.

Therefore, production technology of the green cryo-feed allows satisfy-
ing the need of animals in protein, fatty oils, carbohydrates and vitamins during the entire wintering period. It includes late-summer sowing (mid-end of July) of the most frost-resistant annual fodder crops and their mixtures (oat, oat + pea, oat + rape) with harvesting green mass approximately within 60-70 days (cutting in windrows in September, pressing and warehousing in October) [45, 46]. Cold-preserved perennial sown and natural meadow grass may be used for the same purpose. Wintering of Yakut horses adapted for free year-round pasturing is also possible in such circumstances [55, 56].

Preeminence of cryo-feed was established upon comparative zootechnical assessment of after-grass productivity in essential grass stand and late sowing of oat for making of cryo-feed at wintering of horses [57]. The best digestibility of nutritional substances in winter grass mass of oat as compared to grass stand after-grass is due to high content of carotene and provitamin E in plants preserved by the natural cold during vegetation. Feed capacity of 1 ha with the cryo-feed at wintering of adult animals had comprised 129-142 days per head. To this end, supportability of exchange energy with a view to 100 kg of body weight reached 32.6 mJ, which exceeds the standard value by 14.5 %, consumption of the digestible protein comprised 242.4 g, which exceeds the standard value by 31.0 %. During inspection of a mare breeding stock fed by cryo-feed in December-January, a 30-day outcome of born live foals made 72 % that is 12 % higher than that in the control group. In general, economic effectiveness comprised 2.18 rubles per 1 ruble of costs [57]. High effectiveness of oat winter pasturing from December to February (85 days) was revealed for young Yakut horses. In the trial group, body weight gain in animal, as compared to the control group fed by hay in combination with wintering at natural pastures, comprised 16.9 kg per heads. Cost of 1 dt of the body weight in young animals in the trial group was 51.2 % lower than in the control one. Accordingly, use of frozen oat crops sown during summer is effective, absolutely harmless for young horses, and ensures gaining of body weight during the coldest winter months. Studies had shown changes of several biochemical blood parameters of young animals signifying improvement of protein, fat, and carbohydrate exchange in the trial group, which allows such method for wide use in horse herd farming [58-60].

It appears that specific role of green cryo-feed FFA in regulation of adaptation in the herbivores to cold climate in cryolithozone of Yakutia is associated with effect on thermogenesis in mammals under the long-term low-temperature stress, fulfilling functions of the main substrate for oxidation in mitochondria and disconnecting respiration and phosphorylation [29, 30].

The studies allowed us defining for the first time the general theory of resistance mechanisms in plants and animals to long-term hypothermia in conditions of Yakut cryolithozone [61-65].

Thus, adaptation of plants to long-term low-temperature stress is closely related to main energy sources — lipids and polyenoic fatty acids playing the main role in formation of the high nutritional value in green cryo-feed, the naturally frozen plants in cryolithozone. Green cryo-feed protects vital activity of herbivorous animals, including agricultural species (Yakut horse, reindeer, etc.), in conditions of long-term extremely cold winter. For agricultural production in cryolithozone, it is important to apply special resource and energy saving technologies adapted for local properties.

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