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4.1.2. Селекция, семеноводство и биотехнология растений (биологические науки)

ВЫСОКОЛИЗИНОВАЯ КУКУРУЗА: ПАМЯТИ АКАДЕМИКОВ В. Г. РЯДЧИКОВА И М. И. ХАДЖИНОВА

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В 2024 г. исполняется 125 лет академику ВАСХНИЛ Михаилу Ивановичу Хаджинову и 90 лет академику РАН Виктору Георгиевичу Рядчикову. Они были в СССР пионерами в решении проблемы усиления питательной ценности зерна кукурузы селекционным путём. В основе их работ лежали эффекты мутации гена opaque-2, механизм действия которой был выявлен в США 60 лет назад. Многолетние исследования в Краснодарском НИИСХ показали особенности метаболизма созревающего мутантного эндосперма кукурузы: мРНК бифермента лизинкеторедуктазы-сахаропиндегидрогены, превращающему в клетке свободный лизин в глутамин, не стабильна и легко уничтожается повышенной активностью РНК-азы, это приводит к накоплению свободного лизина в эндосперме, который ядовит для живой клетки. Для нейтрализации лизина усиливается синтез лизинбогатых белков альбуминов и глобулинов, которые являются ферментами, определяющими метаболизм и дифференциацию тканей эндосперма. Как следствие нарушается формирование роговидной (периферической) ткани, определяющей механическую прочность зерна, и эндосперм оказывается представлен только центральной мучнистой тканью, легко поражаемой микроорганизмами и грибами. Это так называемый «высоколизиновый синдром». Исследования в лаборатории показали, что он практически идентичен «адаптационному синдрому», картине биохимических изменений метаболизма в растительной клетке при закаливающей зоне стресса, когда повышена активность РНКаз. Работы американских и французский ис-

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4.1.2. Plant breeding, seed production and biotechnology (biological sciences, agricultural sciences)

HIGH LYSINE CORN: IN MEMORY OF ACADEMICS V. G. RYADCHIKOV AND M. I. KHAJINOV

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In 2024, Academician of the All-Union Academy of Agricultural Sciences (VASKhNIL) Mikhail Ivanovich Khadzhinov would be 125 years old, and Academician of the Russian Academy of Sciences Viktor Georgievich Ryadchikov would be 90 years old. They were pioneers in the USSR in solving the problem of improving the nutritional value of corn grain through selection based on the mutation of the regulatory gene opaque-2, the biochemical effect of which was discovered in the USA 60 years ago. Long-term studies at the Krasnodar Research Institute of Agriculture have shown the peculiarities of the metabolism of the maturing mutant endosperm of corn: the mRNA of the bienzyme lysine ketoreductase-saccharopine dehydrogens, which converts free lysine into glutamine in the cell, is not stable and is easily destroyed by the increased activity of RNase. This leads to the accumulation of free lysine in the endosperm, which is toxic to living cells. To neutralize lysine, the synthesis of lysine-rich proteins, albumins, and globulins, which are enzymes that determine the metabolism and differentiation of endosperm tissues, is enhanced. As a result, the formation of horn-like (peripheral) tissue, which determines the mechanical strength of the grain, is disrupted, and the endosperm is represented only by central mealy tissue, easily affected by microorganisms and fungi. This is the so-called “high-lysine syndrome.” Laboratory studies have shown that it is almost identical to the “adaptation syndrome,” a pattern of biochemical changes in metabolism in a plant cell during a hardening stress zone, when RNase activity is increased. The work of American and French researchers using the microchip method showed the vaследователей методом микрочипов показали справедливость этого вывода. Повышенное содержание в зерне мутанта катионов магния и снижение степени полиаденилирования мРНК позволило сформулировать молекулярно-физиологическую гипотезу о молекулярных механизмах формирования морозоустойчивости пшеницы и ячменя

lidity of this conclusion. The increased content of magnesium cations (Mg++) in the grain of the mutant and the decreased degree of mRNA polyadenylation made it possible to formulate a molecular physiological hypothesis about the molecular mechanisms of the formation of frost resistance in wheat and barley

Ключевые слова: ЗЕРНО, ГЕН OPAQUE-2, МУТАЦИЯ, РНКАЗА, ВЫСОКОЛИЗИНОВЫЙ И АДАПТАЦИОННЫЙ СИНДРОМЫ

Key words: GRAIN, OPAQUE-2 GENE, MUTATION, RNA-ASE, HIGH-LYSINE AND ADAPTATION SYNDROMES

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Introduction

It was discovered in the latter part of the 1800s that proteins are made up of amino acids and that the nutritional value of various proteins varies. But only in 1914, after the discovery of essential amino acids by T. Osborne and L. Mendel, it became obvious that the biological value of proteins is determined by their amino acid composition and that protein is necessary for humans and animals not in itself, but as a source of amino acids.

The problem of protein remains acute in Russian livestock farming and can be solved through its production and rational use. There is an acute deficiency of lysine and tryptophan in food and feed, which are essential amino acids for humans and monogastric (non-ruminant) animals with a single-chamber stomach.

Corn grain provides 15% of the protein and 20% of the calories in the world's food supply. It is digested by animals better than other grains (by 90%), while being the highest in energy and calories. However, its nutritional value is not high due to the very low content of the above-mentioned essential amino acids in the storage protein (zein).

The creation of a highly nutritious protein complex of corn grain still remains a global problem and is being solved by modern methods of genetic engineering. But in our country in the 1970s of the 20th century, enthusiastic pioneers in solving this problem at the Krasnodar Scientific Research Institute of

Agriculture (KNIISKH) were M.I. Khadzhinov (Fig. 1) and V.G. Ryadchikov (Fig. 2), who will turn 125 and 90 years old in 2024, respectively.

Figure 1 – Mikhail Ivanovich Khadzhinov (1899-1980) – geneticist and breeder, student of N. I. Vavilov. Academician of VASKhNIL (1966). Even before moving to Kuban (1940), the author of major monographs on the biology and selection of corn, the scientist’s achievement was the discovery of the phenomenon of cytoplasmic male sterility (sterility), that is, the absence of normal pollen in corn panicles (sultans) of corn (1931) regardless of the American researcher M. Rhodes. Hero of Socialist Labor (1966), Lenin Prize (1963), awarded four Orders of Lenin (1966, 1971, 1973, 1979), Order of the Badge of Honor (1957), medals. He was awarded the honorary title “Honored Scientist of the RSFSR.” In 1972-1977 - vice-president of the All-Union Society of Genetics and Breeders named after N. I. Vavilov. Since 1977 - president of the corn and sorghum section of the European Association of Geneticists and Plant Breeders.

On the occasion of the 90th anniversary of Academician of the Russian Academy of Sciences V. G. Ryadchikov, a book of his memoirs was published entitled “Confession of a Livestock Technician” [4], which, in particular, describes the events associated with breeding and biochemical work on the creation of high-lysine corn based on the biochemical effect of the regulatory mutation the opaque-2 gene, discovered in the USA in 1964, i.e. exactly 60 years ago [10]. Therefore, it is possible to experience the basic emotional and evaluate the scientific aspects of this large research and production project, which formed part of the global search for ways to improve the nutritional value of corn grain.

The fate of enthusiasts

Mikhail Ivanovich Khadzhinov. In 1940, Nikolai Ivanovich Vavilov was arrested. 40-year-old Misha Khadzhinov was at a loss, like many of his comrades, Vavilov’s closest collaborators. Their fate was unclear. Misha sat thoughtfully on a chair in the corridor of VIR - the All-Union Institute of Plant Growing, the brainchild of Nikolai Ivanovich.

At this time, a creative person came to VIR from Kuban, puzzled by finding here a specialist in corn breeding, who was clearly missing at the Krasnodar breeding station. This man had interested conversations with the bosses, who were unable to say anything definite on this issue. Suddenly, one of the VIR employees approached him in the corridor and said quietly: “Do you see the young man on the chair? He is small in stature, but very big on corn. Don't ask anyone, just take him to Krasnodar. You will not regret...".

In 1967, academician of VASKhNIL Mikhail Ivanovich Khadzhinov began breeding work to increase the content of the necessary amino acids tryptophan and lysine in corn grain. But for this fundamentally new work, he needed an assistant who could carry out biochemical work to determine amino acids in grain.

Viktor Georgievich Ryadchikov worked at the All-Union Institute of Animal Husbandry in the 1960s. The issue of amino acid imbalance captivated him. Amino acids compete with each other during absorption in the digestive tract and during translation during biosynthesis on the ribosomes of the protein chain. American researchers have shown that excesses of certain amino acids with a deficiency of others cause a sharp decrease in growth, nitrogen use and other changes in physiological functions in white rats.

To study amino acid imbalance, Victor mastered the amino acid analyzer of the Japanese company Hitachi. This was exactly what Mikhail Ivanovich needed for successful work. Therefore, Viktor Ryadchikov was invited to work at the KNIISKh, where, at the age of 35, he headed the protein quality assessment laboratory, which was equipped with the latest science and technology.

Figure 2 – Viktor Georgievich Ryadchikov (1934-2020) – Soviet and Russian scientist, specialist in feeding, physiology and biochemistry of farm animals. Academician of the Russian Academy of Sciences and Russian Academy of Agricultural Sciences (2013, 1995), Doctor of Biological Sciences and professor (1982, 1988). Laureate of the Government of the Russian Federation Prize and Honored Scientist of the Russian Federation (2000, 1994).

Suffice it to say that the laboratory had four amino acid analyzers from the Japanese company Hitachi, two liquid chromatographs from the same company, Technikon and an installation for the determination of lysine and protein with orange-g dye (Sweden). An installation for isofocusing proteins from the USA, a densitometer for studying nucleic acids from the UK. Three ultracentrifuges: two from the German company VAC (601 and 602) and one from the Japanese company Hitachi. Centrifuge Park, spectrophotometer. And much more. The laboratory received chemicals without restrictions from Germany from Merck and from Switzerland from Sigma.

All this was largely a consequence of a visit to the institute in 1968, a year before the creation of the laboratory by V. G. Ryadchikov, General Secretary of the CPSU L. I. Brezhnev and Chairman of the Council of Ministers of the USSR A. N. Kosygin. The purpose of the visit is to get acquainted with the work of the institute on the creation of highly productive grain crops, as well as to express deep respect to Academician P. P. Lukyanenko for his outstanding breeding achievements.

State leaders also talked with Academician M.I. Khadzhinov. He shared his plans for the future and spoke about the importance of creating high-lysine corn hybrids for livestock breeding and human nutrition. Academician P. P. Lukyanenko was also interested in this topic.

Collaboration of enthusiasts. Academician of the All-Russian Academy of Agricultural Sciences M.I. Khadzhinov was the first in the USSR to develop models of forms of highly nutritious corn with a high content of protein, lysine and tryptophan based on a number of mutations that change the metabolism of the endosperm of the corn grain. Mutation of the regulatory gene opaque-2 was the main hope for increasing the content of the necessary amino acids tryptophan and lysine in corn grain. However, an increase in the nutritional properties of corn grain was associated with a decrease in yield and a deterioration in the physical properties of the grain [1, 2].

During combine harvesting, the grain of high-lysine corn was more easily damaged than the grain of regular flint corn. At the time of harvest, this corn had a higher moisture content than normal corn. If it could not be dried immediately to standard humidity, it quickly began to mold. At that time, drying equipment was extremely insufficient on farms, which created difficulties with the preservation of new corn. If it was dried in time, then there were no problems with its storage.

Its nutritional advantage could be observed not only when feeding animals on farms, but also in the field. The birds happily pecked it from the cobs, while ordinary corn in the neighboring field remained untouched [4].

Numerous studies around the world have shown that, under the influence of a mutation in the opaque-2 gene, the activity of many enzymes changes [1, 2]. In particular, the mutant endosperm contains less starch, which is associated with low activity of the enzymes glucose-6-phosphate ketoisomerase and ADPG-starch glucosyltransferase (starch synthetase). Starch is very dense and makes up at least 70% of the dry matter in the endosperm. This determines the deterioration of the physical conditions of the grain and the decrease in the yield of high-lysine hybrids. In addition, the increased moisture content of mature grain of new corn, associated with a relatively high content of free amino acids, sugars, and ash elements, determines the increased susceptibility of grain to insects and fusarium.

The theoretical foundation for the idea of producing high-lysine corn was developed in the laboratory of V. G. Ryadchikov in order to get around the challenges that arose. The cause of the pleiotropic effect is increased RNase activity in the ripening grain of high-lysine corn and, as a consequence, a redistribution of mRNA stability, leading to an increase in the synthesis of some proteins but a sharp decrease in the synthesis of other proteins, according to experimental studies conducted to investigate the reasons behind the redistribution of protein fractions in the mutant grain.

In addition to a reduction in the primary zein storage protein's mRNA levels within a mutant maize grain, the content of mRNA of other proteins also changes compared to their level in the grain of normal corn.

A series of studies were conducted in the laboratory of V. G. Ryadchikov to test the idea that the destruction of short-lived RNA, which is responsible for the instability of some mRNAs, leads to the stabilization of the remaining mRNAs. It was demonstrated that when actinomycin D blocks transcription, the ripening grain of regular corn's mRNA decays, increasing the grain's lysine content, and RNase inhibitors introduced into the cob of high-lysine o2-corn reduce the lysine content in its grain compared to the control.

All biochemical changes in the grain of the mutant for the opaque-2 gene were theoretically explained by the paradoxical effect of an increased level of RNase activity on the mRNA population.

It was experimentally established that the mRNA of the major components of zein 22 kDa is less stable than the zein 19 kDa mRNA, and the mRNA of the bienzyme lysine ketoreductase-saccharopine dehydrogenase (LKR-SDH), which converts free lysine into glutamine in grains, is characterized by very weak mRNA stability. This corresponds to the molecular biological picture observed in the ripening grain of mutant corn: the mRNA of the bienzyme lysine ketoreductase-saccharopine dehydrogens is very easily destroyed by increased RNase activity, this leads to the accumulation of free lysine in the endosperm, which is toxic to the living cell. Therefore, to neutralize lysine, the synthesis of lysine-rich proteins, albumins and globulins, is enhanced. But these proteins are enzymes that determine the metabolism and differentiation of endosperm tissues. As a consequence of their inadequate activity, the formation of horn-like (peripheral) tissue, which determines the mechanical strength of the grain, is disrupted, and the endosperm is represented only by central mealy tissue, easily affected by microorganisms and fungi [1, 2].

Biochemical and molecular biological research

The ripening grain of mutant corn is a pioneer plant object in important fundamental research that allows us to obtain new knowledge about the regulation of gene expression, which, in turn, involves the development of new genetic engineering approaches to increase its nutritional value.

Global genetic engineering research has led to the isolation, sequencing and study of the regulation of gene expression of corn grain storage proteins - zeins, as well as the opaque-2 gene itself [7, 12].

At the Institute of Bioorganic Chemistry of the USSR Academy of Sciences, the Soviet scientist Vladimir Alekseevich Efimov (1947-2010) synthesized the zein gene, enriching it with lysine codons [1, 2]. Unfortunately, this joint work with KNIISKH was interrupted by the processes of perestroika in our country.

Researchers from around the world have devoted many years to studying the genetic and biochemical phenomenon of mutation of the opaque-2 regulatory gene. An outstanding researcher of this mutant at the molecular biological level is the American scientist Brian Larkins (Brian Larkins). His first work on corn dates back to 1975, and his last one is 2022.

Currently, this problem is being studied using a combination of research methods: transcriptome (cDNA libraries with mRNA), proteome (proteins that are expressed in a given cell, tissue, or organism at a given time) and metabolic analysis (methods of mathematical modeling of metabolism, which allows one to determine the rate of reactions in the metabolic network) [6-9, 11, 12].

On the relationship between high-lysine and adaptation syndromes

Analytical work in the laboratory of V. G. Ryadchikov based on literature data showed that under extreme environmental factors corresponding to the hardening stress zone, when the level of RNase activity is increased, plant cells develop a complex of biochemical changes (adaptation syndrome), similar to that in high-lysine mutants (high-lysine syndrome): changes in the activity of many enzymes [1, 2]. This hypothesis was experimentally confirmed by B. Larkins using the microarray method [9].

According to the laboratory of V. G. Ryadchikov, the endosperm of ordinary corn consists of two tissues that differ radically in the level of RNase activity: peripheral, horn-like (12 units of activity per gram) and central, mealy (60 units/g). At the same time, mealy tissue is characterized by all the biochemical features of the high-lysine syndrome, like endosperm, mutant for the regulatory gene opaque-2: RNase activity is 5 times higher, increased lysine content associated with the redistribution of protein fractions - reduced content of the main storage protein - zein, poor in lysine, but the content of albumins and globulins rich in lysine is increased; starch synthesis is impaired [1, 2]. The endosperm of the mutant consists entirely of mealy tissue. French researchers using microchips confirmed that powdery tissue is formed under hypoxic conditions, that is, under stressful conditions [6].

Perhaps the increased content of enzymes (albumin and globulins) determines the increased 2.5-times level of magnesium cations (Mg2+) in the mutant grain, because magnesium activates over 300 enzymes and is involved in a wide range of metabolic processes. Magnesium regulates the synthesis of cyclic AMP and is involved in the creation and degradation of nucleic acids, proteins, fatty acids, and lipids. At the same time, the degree of mRNA polyadenylation in the mutant endosperm is reduced: the mRNA of protein bodies, microsomes, and free polyribosomes in ordinary corn is correlated in this indicator as: 0.88; 0.55; 1.06; and in the opaque-2 mutant: 0.75; 0.36; 0.16. [1, 2].

Cations magnesium is an important component during the creation of the spatial structure of RNA and its stabilization. The most general effect of Mg2+ on any tissue is that it stabilizes the structure of ribosomal and transfer RNA, but destabilizes the mRNA molecules that control the overall rate of protein synthesis [3, 5].

These facts about the ratio of magnesium content in a cell and the degree of polyadenylation of its total mRNA, obtained in the investigation of the high-lysine syndrome of the opaque-2 mutation, are of fundamental importance during the creation of the adaptation syndrome. In particular, in the development of molecular physiological concepts about the formation of frost resistance of winter varieties of wheat and barley.

When studying the phenomenon of frost resistance, it was shown that the more magnesium in ribosomal RNA, the higher the translational activity of polyribosomes in vitro. That is, it can be assumed that translational activity is determined by two parameters: the degree of polyadenylation of mRNA and the amount of magnesium cations in rRNA [3].

This hypothesis (Fig. 3) requires additional research, but there is no doubt that studies of the relationship between high-lysine and adaptation syndromes have provided an experimental and theoretical basis for its formulation.

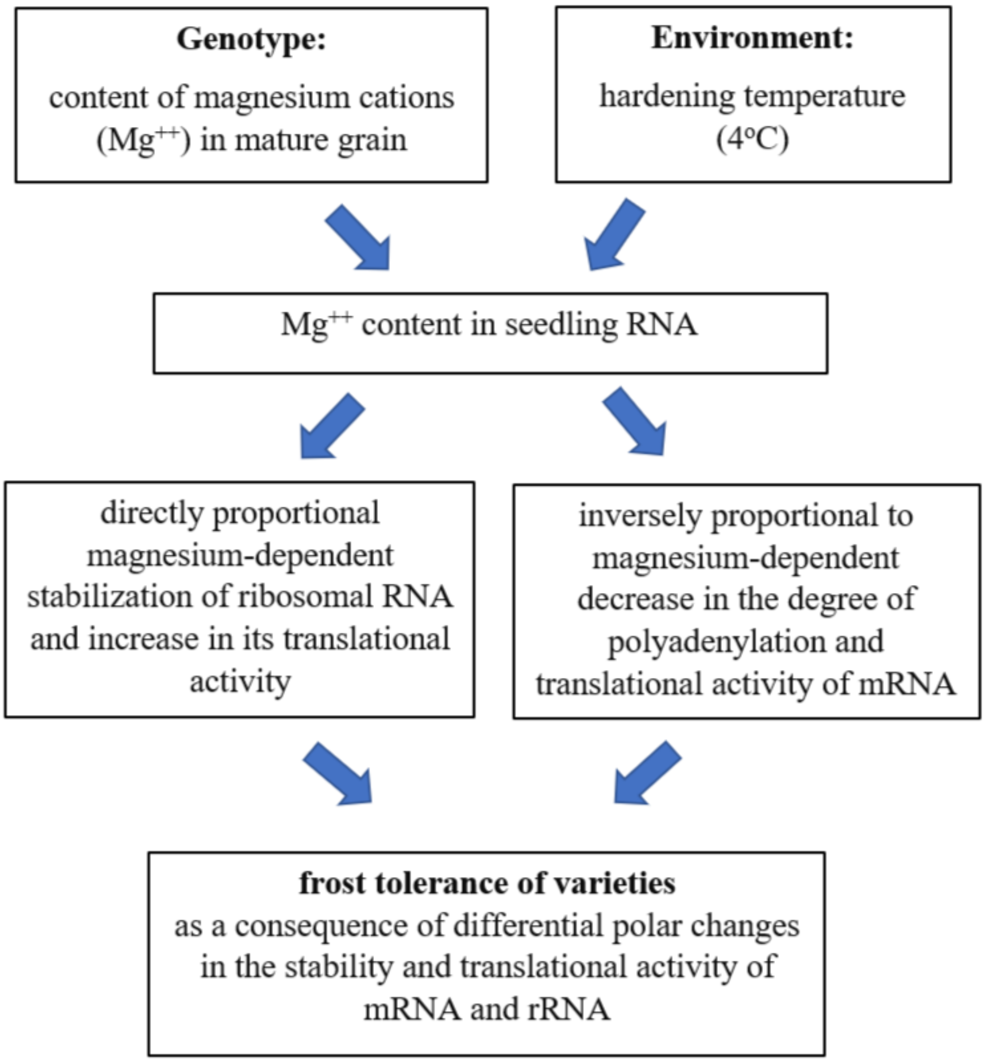

Figure 3 – The concentration of magnesium cations in the cell is a trigger for the formation of frost resistance of winter forms of soft wheat and barley (hypothesis)

Thus, the 1968 visit of L. I. Brezhnev and A. N. Kosygin to the KNIISH not only supported breeding work, but also provided fundamental research on the molecular physiology of ripening corn grains for Academician M. I.

Khadzhinov and the frost resistance of winter varieties of wheat and barley for academician P. P. Lukyanenko.

In addition, in the vivarium of V. G. Ryadchikov's laboratory, model experiments were carried out to study the effect of lysine and tryptophan imbalance on the growth and development of laboratory white Wistar rats and piglets. The fundamental effect of the imbalance was to reduce polyadenylation and the corresponding stability of liver mRNA. However, the translational activity of polyribosomes in vitro was correspondingly reduced with tryptophan imbalance, but paradoxically increased with lysine imbalance. The reason is an increase in the content of magnesium cations in the liver due to lysine imbalance [1, 2]. This corresponds to the patterns presented in Figure 3 about the relationship between the stability of rRNA and mRNA when the concentration of magnesium cations changes in the eukaryotic cell.

The successful development of molecular biology in the world opens up new opportunities, new ways in order to address the issue of raising the nutritional value of corn grain. Research on RNA interference and genome editing has gained significant importance recently.

However, the researchers encountered insufficient selectivity towards the target RNA. In the future, when the problems of nonspecific RNA binding are resolved, RNA interference may become the main direction for improving the nutritional value of corn grain. Then the fruits of the activities of academicians M.I. Khadzhinov and V.G. Ryadchikov and their dreams of radically improving the feed supply of Russian livestock will come true. May their memory be blessed…

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