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“Omic tools” for investigation creative plant systems

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Abstract. The result of the genotype/environment (G/E) interaction affects the success of the implementation of the genetic program of a plant biological system of any level, from a cell population to a multicellular organism. During this interaction, the plant system absorbs trophic and energy resources, processes and assimilates them. Under normal conditions, signal perception and transduction occurs against the background of homeostasis regulated by the genome. Genetic control is exercised at all stages of growth and development of plant systems via differential gene expression. The activity of metabolism is coordinated by the cooparated action of the ionome, proteome, metabolome, and transcriptome. Direct and cross connections between these aspects of life activity are established and developed constantly and manifest themselves in the form of dynamic phenotypic effects from structural formations and enzyme chains. Disturbanses within the individual stages of metabolism and the disconnection between them reveal differences between stable, sensitive and unstable forms. The obtained information is the basis for experiments to obtain forms with improved characteristics. A range of tasks has been outlined in this direction, and there have already been significant developments. Comparison of the dynamics of the functioning of creative variants of plant systems of any level showed their significant differences from the original forms. Changes in creative systems are determined by the interactions of transgenes with endogenous genes and can manifest themselves in the form of positive/negative/combined characteristics of the new system. Comparative studies of the dynamics of vital activity will provide information about the coordinated process of communication both within the cell and between the tissues of a multicellular organism.

The use of various combinations of “omic tools” will facilitate the discovery of new promising candidates among structural and regulatory genes, as well as among promoters. On the other hand, the obtained biological information will be a stimulus for improving the methods and directions of research.

Key words: ionomics; proteomics; metabolomics; transcriptomics; biological systems.

«Омічні інструменти» для дослідження креативних рослинних систем

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Резюме. Результат взаємодії генотип/середовище (Г/С) впливає на успішність реалізації генетичної програми рослинної біологічної системи будь-якого рівня – від популяції клітин до багатоклітинного організму. Під час цієї взаємодії рослинна система поглинає трофічні та енергетичні ресурси, переробляє та асимілює їх. За нормальних умов сприйняття і передача сигналів відбувається на тлі гомеостазу, що регулюється геномом. Генетичний контроль здійснюється на всіх етапах росту і розвитку

рослинних систем через диференціальну експресію генів. Активність метаболізму координується кооперованою дією іономау, протеому, метаболому і транскриптому. Прямі та перехресні зв'язки між цими аспектами життєдіяльності встановлюються і розвиваються постійно і проявляються у вигляді динамічних фенотипових ефектів з боку структурних утворень і ферментних ланцюгів. Порушення в межах окремих етапів метаболізму та розрив зв'язку між ними виявляють відмінності між стабільними, чутливими та нестабільними формами. Отримана інформація є основою для експериментів з отримання форм з покращеними характеристиками. У цьому напрямку окреслено коло завдань, і вже є значні напрацювання. Порівняння динаміки функціонування креативних варіантів рослинних систем будь-якого рівня показало їх суттєві відмінності від вихідних форм. Зміни в креативних системах визначаються взаємодією трансгенів з ендогенними генами і можуть проявлятися у вигляді позитивних/негативних/ комбінованих характеристик нової системи. Порівняльні дослідження динаміки життєдіяльності нададуть інформацію про скоординований процес комунікації як всередині клітини, так і між тканинами багатоклітинного організму.

Використання різних комбінацій "омічних інструментів" сприятиме відкриттю нових перспективних кандидатів серед структурних і регуляторних генів, а також серед промоторів. З іншого боку, отримана біологічна інформація стане стимулом для вдосконалення методів і напрямів досліджень.

Ключові слова: іономіка; протеоміка; метаболоміка; транскриптоміка; біологічні системи.

INTRODUCTION

Planning and conducting of any scientific research involves organizing of a systematic approach with a detailed study of individual components that combine the static and dynamic characteristics of objects of interest. Biological objects, including plants, are the most complex ones, since they are open systems through which flows of substances and energy floods in different directions. At the same time, they are separated from the environment by structures that minimize trophic and energy losses and serve to maintain the spatial integrity of the system. Such isolation takes place both at the cellular level and in multicellular organisms [1, 2, 3]. At the cellular level, various compartments are separated within the cell, which allows to form the direction of metabolism and its products. Due to this, unicellular organisms with different characteristics can successfully coexist in the same conditions. In multicellular organisms, separation occurs at several levels: tissues - organs - organ systems – entire organisms. This segregation is effective both under normal conditions and under stress. The vital activity of biological systems of any rank is supported in this way, subordinating them to their common goal - self-reproduction and distribution. Both unicellular and multicellular forms realize their genetic potential to the maximum for this purpose. Differential gene expression is manifested by phenotypic genotype/ environment (G/E) interaction reactions [8, 12].

MATERIALS AND METHODS

Modern assessment methods made it possible to compare the development of plant cenoses of different ranks by analyzing and comparing a multicellular organism and a natural unicellular system, as well as a cell culture obtained from differentiated tissues. The evaluation takes place under different cultivating conditions. In adequate situations, there are differences in the expression of the genes of the whole organism with the cooperative functioning of organs and the cell population, in which its components (cells) develop independently. Under normal conditions, optimal reactions occur; those reactions stabilize homeostasis. The roles of stability mechanisms of various hierarchical levels are established under stress pressure [4, 47, 48]. For example, links in the chain of their synthesis can be traced when studying secondary metabolites [5]. Cytogenetic analysis reveals the points of influence of the agent on the genetic apparatus [6].

RESULTS

Aims of the investigation; modernization/ establishment of experimental approaches.

Scientific aspirations have always been subordinated to the interests of man. The population is constantly increasing. The ongoing changes often have a critical impact on the environment, which leads to significant climate changes.

Recently, the biodiversity of plant systems has undergone significant transformations. On the one hand, natural cenoses are significantly impoverished. In many cases, they completely disappear. On the other hand, there is a significant variety of forms that did not previously exist. In this regard, the problem of introducing new genotypes into the natural habitat arises.

At present, various biological methods for obtaining new forms of plants exist and are constantly being improved. In parallel with traditional approaches such as hybridization, mutagenesis or polyploidy, new scientific ideologies and strategies are being developed. At the same time, modern alternative methodologies for the "development" of plants, in comparison with traditional ones, are designed to be more "technological" due to technical unification, and their results / products turn out to be creative. In a significant number of cases, new plant forms are completely designer, since the introduced modifications can cover both structural and regulatory genes, as well as promoters that play a similar role, which makes it possible to optimize the communication of different compartments with changes in external factors [47, 48, 156].

The G/E interactions of creative organisms can differ significantly, even radically, from those expected in any direction. In this regard, it is advisable to consider genetic modifications not as a discrete event, but as a component of the entire metabolism. This approach will make it possible to identify unknown genes involved in the process which is studied.

For example, populations of mutants identified as salt/osmo/temperature/ABA responsive were obtained only in Arabidopsis [7, 9]. In general, there were tested more than 250,000 (!) lines with independent insertions and more than 200 individual mutants, in which

modifications of stress reactions were noted. Stress-responsive phenotypes have been noted in the case of mutations in genes involved in many basic cell functions associated with a wide range of cellular homeostasis mechanisms.

Transgenic plants and progeny were analyzed [9, 10, 17]. Two genetically salt-tolerant mutants were isolated from 11000 lines of the T2 generation. One of them, sto1, has been characterized. Salt tolerance was due to an insertion in the *NCED3* gene, which encodes the isoform of 9-cis-ethoxycarotenoid deoxygenase, an enzyme catalyzing the critical site of ABA synthesis under stress. The plant was evaluated in terms of root morphometry, anthocyanin accumulation, and stomatal closure during dehydration. At the same time, it is obvious that changes in any compartments are based on indicators of dynamic processes covering all aspects of the metabolism of new plant forms. First, these indicators, recorded in situ, are the result of the realization of the genotype. Secondly, they are carried out thanks to numerous direct and cross paths of interactions. Thirdly, they are dynamic enough in themselves to respond to changes in external conditions adequately.

In conclusion: the recognition of this postulate led to the identification of separate areas of research, namely, transcriptomics, proteomics, metabolomics, and ionomics. In some cases, approaches united by the general term “omic tools” are being developed/improved for this purpose.

Ionome and ionomics.

In general, an ionome is defined as the composition of macro- and microelements in a plant and is an inorganic component of cellular and organismal compartments. The ion pool in plant systems is organized as a result of external inputs. Inorganic components after permeation can act as part of the active structures of the organism or accumulate in an inactive form. In the first case, a metabolic pool is formed under normal conditions. In the second case, we can even talk about hyperaccumulation [11]. Such an event takes place for several ions and in a stressful situation.

The ionome of plant systems under normal conditions has a similar qualitative composition of metal and non-metal ions. They include macronutrients (N, C, H, P, O, S) and ions of some metals – potassium, calcium, iron, magnesium, zinc, copper, and molybdenum. In this case, metal ions can be present both in the form of cations and anions. This circumstance is realized during the formation of the structure and functions of some enzymes (cofactors). In some exceptional cases, the ionome can be replenished or changed due to “non-traditional” elements, but in this case we will talk about peculiar forms.

In quantitative terms, all the elements that make up plants can be divided into three parts: macronutrients - from 10 to 0.01 % of the composition; trace elements - from 0.001 to 0.00001 %; ultramicroelements - 10^{-6} ~ 10^{-12}% [45, 83]. However, a classification that reflects their biochemical physiological functions should be recognized as more appropriate. So, potassium, calcium and chlorine ions take part in maintaining the osmotic status, regulate the electronic balance and affect the permeability and electrical conductivity of membranes [13, 14, 15]. Manganese ions activate kinases, transferases, decarboxylases. Ions can also interact with each other, positively or negatively [15]. When studying the transport of nitrates in the mutant line of Arabidopsis, the fact of nitrogen uptake that was consistent with the transfer of Na^+ ions was established [16].

Using the Arabidopsis model object, the ionome is analyzed in different directions. Over the past decade, 205169 Arabidopsis specimens have been tested for the presence of 18–20 elements. There were 40388 wild-type plants and 28357 T-DNA mutants among the probands; 3452 genes, 1474 inbred lines, and 753 natural associations were studied [16, 18, 19]. Measurement of the component qualitative composition and the dynamics of its changes, as well as the relationship with genetic elements, can be an alternative way of analyzing genes and genetic networks [20].

The first ionomic mutant *esb1-1* had an increased content of suberin (***e**nhanced **s**uberin*) in the roots [21]. These morphological features disturbed the normal deposition of lignin and prevented water transport. A lot of mutants exhibit phenotypic changes which are associated with changes in the ionome [22, 23, 24]. Thus, the *NaKR1* gene encodes a metal-binding protein that is required for the phloem. The *nakr-1* mutant was characterized by increased accumulation of Na^+, K^+, and Rb^+ ions [22]. The mutants which were associated with changes in the content of molybdenum were characterized by changes in the integral indicators of productivity [25].

Changes which are associated with the formation of the ionome can be detcted in the structural, functional, and genetic elements. Attention is drawn to the interactions between K^+, Ca^{2+}, Na^+ ions. For example, the content of these ions in genetically modified maize plants were studied. T3 generation was analyzed. It was noted that GMOs contained significantly lower amounts of Ca^{2+} and K^+ relative to the original form. The Ca^{2+} / K^+ ratio in plants was similar [26, 27, 28]. The noted intragroup differences were a consequence of the pleiotropic effect of genes during meiosis.

The nature of absorption, transport, and balance of physiological cations contains information about the functioning of channels, transporters, and pumps operating both within biological systems and during G/E reactions [29, 30, 31]. Thus, when studying double mutants of Arabidopsis, it was found that the AtHAK5 transporter is the only K^+ uptake system at concentrations <0.01 mM; two transporters - AtHAK5 and AtHAK1 - work in the range of 0.01 ÷ 0.05 mM. Higher concentrations include unknown absorption systems [34].

The study is not limited by the ionic composition of model plants. The ionomes of agricultural plants: rice [32, 33] maize [34, 35], barley [36], soybean [37], and wheat [38] are already being analyzed.

The activity of functional groups, namely: phosphorus-containing, nitrogen-containing, is an established fact.

There is information that Ca^{2+} can form complexes with long-chain saturated fatty acids (C_{16} - C_{22}), especially palmitic and stearicThe formation of these complexes at a certain stoichiometric ratio of ion and

acid leads to an increase in the nonspecific membrane permeability [39].

Second messenger precursors are usually highly phosphorylated and enriched in phosphate groups, and if we take into account the result that inositol triphosphate and diacylglycerol are formed from the components of the membrane, then the interaction between the physiological ions K^+ and Ca^{2+} becomes more pronounced [59].

The interaction among functional phosphorus-containing- molecules of the cell matrix is established. In proton/phosphate cotransport, the protons which are used for transport are supplied from the environment by a proton pump. The proton pump is inactive with a small amount (<1.0 μM) of external phosphate. In this case, protons for co-transport are provided exclusively by the environment. The proton pump is activated with an increase in the phosphate content, resulting in acidification of the cytoplasm during proton cotransport [40].

The interaction of ions occurs with the participation of functional groups containing nitrogen. These groups include NH_4^+, NO_2^-, and NO_3^-. The inner pools of these functional groups are very dynamic and can fluctuate even during the day, since their metabolism is carried out with the participation of enzymes that change their daily activity. The absorption and transfer of nitrates is carried out with the participation of tonoplast transporters [41]. When saturated, nitrates can accumulate in the vegetative organs; when deficient, they can be released. (Such an event develops under stress).

The of nitrate and ammonia ratio is important for protecting the organism from ammonia excess.

It is fixed evolutionarily that organic ions and functional groups in plants of various taxonomic variants are involved in similar reactions. In this case, inorganic components usually remain unchanged in their qualitative characteristics, which determines the possibility of interactions. The replacement/addition of some reactive groups by others is based on chemical analogies and biological antagonism (nitrates → chlorates; molybdenum ion → tungsten ion). This dualism is used when creating new forms [42, 43, 46].

Summing up, it can be noted that ionic interaction is, in fact, a wide range of reactions in which they involve as structural and functional compartments. In this case, organic components may not even directly form chemical bonds with an inorganic ion that has entered or acts from the outside. In creative systems, they can significantly affect the integral characteristics, creating a new plant form.

Proteome and proteomics.

At the same time, there is a special class of compounds in living organisms, which, ensures biodiversity. These are proteins. Proteins form the plant proteome. They can be targets for triggering specialized responses in both normal plants and new forms. This circumstance led to the need to form a separate area of research - proteomics. The identification of the complete proteome is difficult because there is a variety of tissues with differential gene expression in them. Thus, in Arabidopsis suspension culture, hundreds (!) proteins were isolated from preparations associated with microtubules. They are regulators of microtubules, kinesins, dynamins, and proteins that are involved in replication, transcription, and translation have been identified among them.

The G/E interaction is also important for proteome evaluation. The colloidal state of proteins correlates with those actions that stabilize the system under changing external conditions: low thermal conductivity, low diffusion coefficients, and high viscosity. Similarly, the variety of processes that take place in biological systems can occur at the required speed only with the participation of enzymes. Enzymes may be involved in central metabolic pathways; they may determine the synthesis of cellular compartments from intermediate products of metabolic pools and convert primary nutrients into compounds that are included in the central pathways of metabolism. Moreover, many specific activities such as cell interaction, cell dynamics, gene activation/repression, mitogenesis, intracellular transport, and component compartmentation are mediated by proteins [44, 50, 52]. At the same time, the same class of proteins can be classified in different ways. For example, histones can be defined as structural polypeptides. But at the same time, they exhibit the properties of regulatory proteins. Permeases are both membrane and transport proteins [50, 160].

Since the biological activity of proteins is determined by their amino acid sequence, a change in the structure of the molecule can significantly affect the enzyme activity [51, 156]. However, the any enzyme will have the same structure of its molecule and exhibit similar activity both in *in vitro* cell culture and in differentiated plant cells. Comparison of the proteomes of various plant forms can establish the direction of selection of genetically modified variants [53, 54, 55, 56].

A separate promising direction of changes in the proteome is site-specific mutagenesis. This method consists in making point changes in a certain place (site) of DNA, which significantly modify the characteristics of proteins. This is especially true in the case of enzymes, especially enzymes that are coordinated with the main metabolic chains. The first successful attempt of site-specific reconstruction was achieved by Wilkinson who modified an enzyme from the bacterium *Bacillus stearothermophilus* in 1984. Since then, many works illustrating the prospects of this approach have been published [57, 60]. For example, the unitary substitution in the DNA structure of *E. coli* made it possible to increase the efficiency of the acylation reaction of aromatic amino alcohols in the synthesis of new generation of β-lactam antibiotics in 250 (!) tymes [58, 61].

It is known that many proteins are involved in protect reactions against various stress factors. Thus, the antioxidant system serving (catalyzing) the detoxification of ROS and peroxides combines SOD, catalase, ascorbate peroxidase, and glutathione reductase. Each of the mentioned enzymes has its own site of localization, which ensures the optimum activity. Different enzyme

isoforms can be encoded by a single gene or by gene families. The antioxidant system is directly related to resistance to abiotic stresses [62, 63, 64]. Therefore, this feature determines the increased interest in obtaining new forms of plants with increased activity of antioxidant enzymes [65, 162].

Proteins are related to the movement of inorganic ions and ionic complexes. Thus, it was found that the enzyme Ca^{2+}-calmodulin-dependent phosphatase regulates the homeostasis of Na^+, K^+, and Ca^{2+} ions. The calcium cation and CaM-dependent calcium-neurin signaling also affect the determinants of salt tolerance. Thus, the expression of calcineurin in transgenic tobacco plants increased their tolerance to salinity [66]. Overexpression of glyoxylase1 (another Ca-binding protein) also increased salt tolerance [67, 163, 164].

The high affinity K^+ transporter HKT1 may be related to the transfer of Na^+ ions. The emergence of **s**alt **o**verly **s**ensitive (sos) mutants is associated with genetic changes in the HKT. Three genetically related loci, which control salt tolerance, have been identified: SOS1, SOS2, and SOS3. The SOS1 locus encodes the Na^+/H^+ antiporter of the cytoplasmic membrane [68, 69, 70]. An interesting feature of this transporter is the significant variability of its characteristics. Mutations that disrupt the functions of AtHKT mitigated the sensitivity to NaCl of seedlings of Arabidopsis mutants *sos1-1*, *sos2-2*, *sos3-1* in the *in vitro* system. At the same time, another type of mutation, on the contrary, increased sensitivity to NaCl in wild type seedlings [10, 71, 72]. Thus, the proteome - ionome interconnection becomes obvious: changes in the structure of the protein molecule lead to a change in the ionome.

In conclusion:The considered examples show that any changes (qualitative or quantitative) in the proteome can significantly affect the implementation of the integral characteristics of the new genotype in genetically modified variants as well as in creative forms. There are not only direct communications, but new relationships between intracellular compartments and between more distant from each other tissues are also mediated. The appearance/modification/disappearance of a number of compounds that are inherent in wild-type forms is recorded. Proteins (proteome) are actively involved in the synthesis of a number of low molecular weight compounds that significantly affect the vital activity of the organism. A separate character of metabolism can be distinguished for each such segregated structure.

Metabolome and metabolomics.

The term “metabolome” was adopted at the end of the 20th century by analogy with the genome, transcriptome, and proteome [68, 69, 70]. Metabolism unites the chains of carbohydrates, lipids, amino acids, purines and pyrimidines exchange. Between these directions and in each of them, the processes of synthesis and decomposition are constantly interconnected, between them a balance is maintained. Plant metabolomes are substantially comparable, with some differences established [73, 74, 75]. Metabolites differ in chemical composition and are often dissociated at the cellular and subcellular levels [71, 72, 76, 77]. Quantitative and qualitative assessments of cellular metabolites make it possible to characterize the dynamic component of the biochemical status of organisms. The aim of metabolomics is to test the complete metabolic profile that is derived from all compartments.

Emphasis is placed on targeted or non-targeted metabolomics when solving a specific problem. In the first case, a qualitative analysis of a limited number of known substances - biomarkers - is carried out. In the second case, a review analysis of all metabolites is performed, including those that have not yet been identified at this time.

There are three groups of known metabolites. The first group includes steady state metabolites. For the most part, these are lipophilic compounds, such as fatty acids and their derivatives. The second group includes growth metabolites; these are oligosaccharides, maltose, fatty acids with short chains and acids with multiple bonds. The third group is represented by activity metabolites. These representatives have two peaks in their profile in the middle of the growth period and an additional peak at the transition to the stationary growth stage [77, 78]. This group includes unsaturated and fatty acids with medium and moderately long chains (C_9-C_{18}), as well as compounds with a variety of biological activities, energy cycle intermediates.

The scale of metabolome research is carried out using a number of technologies, including: gas chromatography, and mass spectrometry, liquid chromatography, nuclear magnetic resonance (NMR). Each method reveals information at different levels. For example, NMR allows the analysis of metabolites in a large volume of plant material, since it is based on the measurement of atoms with a non-zero magnetic moment. Mass spectrometry detects metabolites at the molecular level, while NMR detects them at the atomic level [80, 81]. The analysis of Arabidopsis metabolite profiles, performed using liquid chromatography/mass spectrometry, made it possible to distinguish two thousand different mass signals from roots and leaves, many of which are specialized [74, 152].

Metabolomics, unlike proteomics and transcriptomics, allows direct assessment of the phenotypic response. While an increase in the amount of mRNA in the study of the transcriptome does not always correspond to an increase in the amount of the protein, and the analyzed proteins may be enzymatically inactive in the analysis of the proteome, then metabolites always perform a certain function in the cell [73, 79]. Therefore, they can be considered as the final stage of gene expression [73]. Therefore, the metabolome can be used for genome monitoring. This becomes especially relevant when evaluating genetically modified forms.

Comparison of metabolome pools of various plant forms revealed the features of creative variants. Thus, the fatty acid composition of membranes in gene-knockout cotton varieties contained a higher proportion of unsaturated fatty acids (UFA) and a lower quota of saturated fatty acids (SFA) compared to the parameters noted in the unmodified Cocker-312 genotype [82].

Metabolite profiles were also studied in other genetically modified (GM) objects. For example, a decrease in the rate of photosynthesis in GM potatoes was noted while maintaining the level of sucrose

synthesis. The content of hexose phosphates and triphosphates decreased, but the size of the pool of inorganic phosphorus did not differ from that of the wild type; the ratio of sugar phosphoesters and inorganic phosphorus was significantly lower [83]. In the tubers of genetically modified potatoes with reduced activity of cytosolic phosphoglucomutase (PGM), a decrease in the level of sucrose mobilization and starch accumulation was observed, which was accompanied by short stature [84]. (PGM catalyzes the reversible conversion of glucose-1-phosphate to glucose-6-phosphate).

The enzyme fructokinase catalyzes the phosphorylation of fructose to form fructose-6-phosphate. Transformation of potato varieties '*Desiree*' and '*Record'* with constructs containing sense and antisense sequences of the fructokinase gene under the control of the 35S promoter provoked a change in the amount of the enzyme and its activity [83]. Antisense inhibition of the fructokinase gene led to a decrease in tuber yield. Overexpression of the gene in the variety 'Record' caused an increase in the pools of alanine, ornithine, and proline.

Changes in amino acid profiles are observed in many genetically modified forms [85, 86]. At the same time, correlations are being searched between the amino acid pool and general characteristics, such as: stress resistance, productivity, quality [88, 89].

In some cases, the formation of the composition of the metabolome can be an event that determines ontogeny and the implementation of the genetic program of the organism. Thus, the reduction of the vernalization period or its complete absence turns winter wheat into "grass by grass" [87, 90].

Metabolism of some amino acids in genetically modified variants significantly affects a number of basic characteristics (resistance) [91, 92].

In some cases, low molecular weight compounds, which make up the vast majority of the metabolome, can affect the synthesis/accumulation of each other. For example, proline and carbohydrates show a very complex relationship [93, 94, 165, 166].

Unlike the genome and transcriptome, which consist of linear polymers of four nucleotides, or the proteome, which consists of 22 primary amino acids, the metabolome includes a vast number of components of various chemical nature. The current method/methods are not able to quantify all components in a single sample. At the same time, the results obtained at this stage of technology development can provide answers to many questions. And genetically modified variants play an important role here.

Thus an analysis of publications has shown that testing of creative organisms should be done systematically, assessing the qualitative and quantitative state of low-molecular compounds as accurately as possible. Profiling of metabolites reveals their individual contribution to the characterization of phenotypes; reveals G/E interactions as well as interactions between individual genotypes. In this case, not only the impact of the environment on the new genotype is assessed, but also the impact of the creative system on the surrounding ecosystem; problems and risks are identified. At the same time, stress reactions and adaptation reactions are differentiated; biochemical markers of structural and functional rearrangements and changes in enzyme activity, are established. Here, the main emphasis is made on targeted metabolomics answering a specific question within an established hypothesis. The fact of genetic regulation/management of the course of metabolism becomes obvious.

Transcriptome and transcriptomics.

Studies of the ionome, metabolome, and proteome of plants characterize the features of their structure, functioning, and ontogenesis. However, the question of regulation by processes remains open with this approach; the cause of the work, the results of which are observed, remains uncovered.

At the same time, in the course of evolution, a genetic system in plants that coordinates the spatial and temporal components of physiological activity was formed. This is a system of transcription factors (TFs, trans-acting factors). TFs are also called master regulators [96, 97, 98].

TFs are proteins or their complexes that are not directly involved in the catalytic synthesis of RNA, but are essential for the performance of all stages of gene transcription by the RNA polymerase enzyme and ensure the selectivity of the process. After entering the nucleus, TFs regulate transcription by interacting in a special way with DNA or another protein that can form a "protein-DNA" complex corresponding to a certain DNA sequence. TFs provide integration centers by acting as effectors of multiple signaling cascades and therefore serve levels of regulation that often involve post-translational modifications. Such modifications can regulate almost all TF functions, including subcellular localization, stability, interaction with cofactors and other post-translational modifications and transcriptional actions quickly and reversibly [7, 84, 96]. TFs are specifically coordinated with the *cis* regions of promoters of regulatory genes with stimulating their activity. This type of transcriptional regulatory system is called a regulon (gene networks).

According to the functional characteristics, three classes of TFs are distinguished. The first one unites the main TFs that provide an unregulated basic level of transcription and work in all cell types. The second one is represented by specific TFs that interact with certain DNA nucleotide sequences. They are the main regulators of transcription and ensure the specificity of gene expression in space and time. The third one includes a group of co-activator proteins that act in cooperation with the main TFs and organize finer regulation of gene transcription. TFs, unlike most structural genes, tend to control several steps in a process. Therefore, they have become an active tool for manipulating the complex metabolic pathways of plants [91, 92].

The key goal of transcriptomics is the identification of the most of/all transcripts, including mRNAs, non-coding RNAs, and miRNAs, in order to establish the transcriptional status of genes, to determine the 5' and 3' ends of the genome, and for post-transcriptional

modifications [99, 100, 101]. The result of the studies can be quantitative indicators of the transcriptome (complete list of transcripts) in accordance with the stage of development [99, 102].

Transcriptomics is directly related to modern biotechnologies. It is actively involved at all stages of obtaining new forms of plants, from designing an experiment to testing the "finished product" and identifying new direct and indirect relationships in them. In a significant number of cases, new plant forms are completely designer, since the introduced modifications can cover both structural and regulatory genes, as well as promoters that play a similar role, which makes it possible to optimize the communication of different compartments with changes in external factors.

At the moment, it has already been established that TFs may be related to key indicators of nitrogen and carbon metabolism [103, 104, 105]. Relationships between TF and genetic changes in the profiles of the ionome, proteome, and metabolome have been established. Thus, it is assumed that the efficiency of phosphorus use (PUE, P-use efficiency) can be significantly increased in GM forms with the help of TF [106, 107]. Sequence analysis of the A (NF-YA), B (NF-YB), C (NF-YC) subunits of the CCAAT-binding nuclear transcription factor (NF-Y) of wheat (*Triticum aestivum* L.) was performed; the reactions to the presence of phosphorus in seedlings were studied [106]. The expression of most NF-YA genes responded to the low availability of phosphorus positively. On the contrary, overexpression of *TaNFYA-B1* on chromosome 6B induced by low phosphorus content significantly increased its uptake [109, 107].

A significant number of confirmations have been obtained regarding the involvement of TFs in maintaining/increasing stress tolerance [110, 11, 112]. Molecular genetic studies have shown that genetic programs that are accompanied by differential expression of genes coordinated with various functions, including those associated with resistance to salt and water stress (osmotic effects), critical temperatures, and heavy metal ions, are implemented in response to the action of many stress factors. [114]. In general, these genes are classified into two groups. One of them includes genes, the products of which are directly involved in the physiological and biochemical processes that are associated with an increase in the level of stress resistance. The second of them combines the TFs genes that regulate the expression of the former [113, 114]. *Trans*-acting factors can be both positive and negative regulators of structural genes. Therefore, the study of *cis*-elements and *trans*-acting factors is aimed at revealing the molecular mechanisms of these processes.

Currently, TFs that are coordinated with stress resistance in one way or another are combined into various families and subfamilies: AR2/ERF, NAC, MYB, MYC, bZIP, NF-Y, Cys2His2 zinc-finger, WRKY [95, 115, 116]. A significant number of publications are devoted to the consideration of morphological, physiological, biochemical, and molecular aspects of the functioning of GM forms of plants that are coordinated with the relationship between TF vs stress tolerance. It is important to note the fact that directions are distinguished in this case: TF-ionomics, TF-proteomics, and TF-metabolomics.

It is well known that jasmonic acid (JA) affects the productivity and resistance of plants [62, 64]. The JIN1/MYC2 protein is one of the key TFs involved in the realization of many JA effects [62]. It is assumed that it affects the FA, the dependent regulation of the expression of genes involved in plant resistance to oxidative stress. It was found that *jin*1 (jasmonate insensitive 1) mutants were more sensitive to the action of oxidative stress producer methylviologen compared to wild type plants [62]. It was also shown that JIN1/MYC2 is involved in the regulation of the expression of genes for monodehydroascorbate reductase, ascorbate and cysteine synthesis enzymes [117, 118]. In addition to being involved in the regulation of gene expression of antioxidant enzymes, TF JIN1/MYC2 is involved in the control of the synthesis of tocopherol and flavonoids [123]. The activity of TF MYB8 is associated with the synthesis of flavonoids. Overexpression of *SbMYB8* caused an increase in the drought resistance of creative forms in tobacco plants that were transformed with the gene of the medicinal plant *Scutellaria baicalensis* [119]. (It is believed that the antioxidant properties of flavonoids are manifested in their ability to chelate metal ions involved in radical processes) [120].

The relationship between TFs and stress resistance can be traced in the analysis of a number of low molecular weight compounds - compatible osmolytes. First of all, this refers to free *L*-proline. To date, a significant amount of information has been collected that confirms the polyfunctionality of this amino acid reliably [5, 121, 122]. On the other hand, *L*-proline is distinguished by structural features of the molecule, which form their own pathway of metabolism/transport/accumulation [3, 121, 122, 123]. These circumstances stimulate activity in the direction of versatile manipulation of free *L*-proline metabolism genes to obtain forms with an increased level of resistance to a variety of stress factors. In this regard, various transcription factors are being investigated.

Thus, it was found that TFs ERF (**e**thylene-**r**esponsive **f**actor) DREB (**d**ehydration **r**esponsive **f**actor) are associated with the maintenance of drought resistance. Transgenic wheat plants expressing Arabidopsis genes were characterized by increased drought tolerance [125, 126]. Changes in the expression of DREB/CBF factors affected the level of resistance in wheat and barley. This was accompanied by an increase in the level of soluble sugars [123, 127, 128].

There is evidence that the expression of transgenes encoding TFs of the DREB subfamily may be accompanied by the induction of transcription of genes of other types of transcription factors [107, 127, 129]. Among these genes, one can point to *STZ*, which is supposedly further involved in signal transduction pathways and in the control of expression of stress-induced structural genes. In addition, this TFs is supposed to be involved in molecular mechanisms affecting the growth rate of transgenic forms [129]. Changes in the transcription patterns of regulatory genes

even under normal conditions could cause a slowdown in the growth rate.

The increased resistance of plants with functional *DREB* genes was coordinated with some improved physiological and biochemical characteristics under stress conditions. However, negative changes could occur in GM plants under normal conditions, along with the absence of differences in growth parameters between transgenic and conventional forms [127, 130, 131]. Significant ectopic expression of the transgene under normal growing conditions could slow down the growth rate, which led to dwarfism and delayed flowering of plants.

Some information about the effect of DREB - transgenic factors on productivity. An increase in the yield of transgenic maize with overexpression of *TsCBF1*- the halophyte *Thellungiella halophila* gene - was noted [131]. In transgenic plants with a functional maize gene *Zm CBF3*, the yield did not differ from the control yield, while yield decreased in transgenic wheat and barley plants overexpressing TaDREB2 and TaDREB3 [127, 132].

The association with stress resistance is demonstrated by the subfamily of ABA-dependent TFs (AREB/ABF; **A**BA **R**esponsive **E**lement **B**inding protein/factors). This subfamily belongs to the bZIP (**b**asic **l**eucine **zi**pper) TF family, which is involved in stress-induced signal transduction. Two areas, differing in the nature of the produced action, are distinguished in the structure: the and the leucine "lightning" (zipper). The conservative part is responsible for sequence-specific DNA binding, while the less conserved zipper region is responsible for the specificity of bZIP-TF dimerization [95, 116, 134]. ABA - inducible expression of structural genes can be carried out in the presence of additional copies of ABRE (**AB**R - **R**esponsive **E**lement, ABA - reacting element) or other "linkers" (CE, **C**oupling **E**lement). CEBFs (CE **E**lement **B**inding factors) TFs interact with CE elements. It is believed that CEBFs are involved in ABA - and sugar-regulated signaling chains. For example, maize TF ZmAB14 interacts with the *cis* element of the promoter of the sugar-responsive gene. Moreover, CE1 can function as an ABRE element, mediating responses to both abiotic and biotic stresses [134, 135].

Information on the genes encoding TFs of the AREB/ABF family has significantly expanded to date. For example, 125 bZIP family genes encoding 170 different bZIP proteins have been identified in the maize genome. The same amount was found in rice [89, 136].

Analysis of the scale of gene expression in a number of biotechnological plants showed that overexpression of genes encoding TFs of the AREB/ABF family can lead to the induction of transcription of structural genes involved in the metabolism of *L*-proline, carbohydrates, and lipids. Transcriptome analysis showed that expression can change in one and a half to two thousand genes. At the same time, the functional role of the majority of AREB/ABF representatives has not been established not only in cultivated plants, but also in the model object, Arabidopsis. A large-scale screening of TFs showed that representatives of many families are related to the formation of stress resistance. Thus, the NAC TF subfamily performs various functions in the processes of growth and development, including participation in the control of responses to abiotic stresses. Thus, in rice, coordination with stress resistance was predicted for 190 genes [136, 137]. Functional analysis revealed the fact that most of NAC TFs are transcriptional activators. These include TFs NAC of wheat, maize, rice, and soybeans [42, 138, 139, 140,]. At the same time, NAC TFs that are inhibitors of transcription are also known. Most representatives of NAC TFs are localized in the nucleus, but can also be located on the membrane.

Transcription factors have an extensive network of relationships with the proteome, ionome, and metabolome. The metabolome has already been mentioned when referring to the study of low molecular weight compatible osmolytes. Proteome/TF relationships are being traced in the development of biotechnologies aimed at manipulating excess late embryogenesis abandoned proteins (LEA). Expression of the *LEA* genes was noted at the late stages of seed maturation, and is also traced in reproductive and vegetative tissues in response to osmotic stress. Thus, dehydrins, one of the LEA groups, prevent the destruction of proteins during dehydration. Transgenic tomato plants with overexpression of their own gene were resistant to salinity and dehydration [141, 142, 143]. Induction of *LEA/COR/DHN* gene expression can occur in resistant plants overexpressing transcription factors. Thus, putative ABA- and stress-responsive *cis*-elements were identified in the promoter region of wheat Cor/Lea genes, which regulate the expression of Cor/Lea *Wdhn*13 *Wdhn*17-18 genes positively [144].

There is also information about the association of TFs with some components of the ionome. Thus, a protein belonging to a unique family of Ca-sensors in plants is known. It is represented in the maize genome by a single copy of ZmCBL4 The maize *ZmCBL*4 gene presumably copies a homologue of the Arabidopsis CBL4/SOS3 protein. Transgenic Arabidopsis plants accumulated less Na^+ and Li^+ ions in comparison with the control [145, 147].

In addition to interacting with the ionome, metabolome, or proteome, TFs of one family can interact with TFs of another family. It was mentioned above that the expression of transgenes encoding TFs of the DREB subfamily was accompanied by the induction of transcription of genes of other TFs [106, 107, 129]. This may be due to various reasons. Among them are interactions with *cis*-elements of promoters, post-transcriptional modification associated with phosphorylation of amino acid sequences. For example, an ABA-activated kinase phosphorylates Ser/Thr residues of RXXS/T sites in the conservative region of proteins of all AREB forms [133, 145].

These publications reflect the coordination of transcription factors with transgenes and endogenous genes within the same organism. At the same time, they are able to influence the creation of complex systems.

Thus, transcription factors are involved in the formation of plant-bacterial symbioses. For example, TF NIN is involved in the activation of *CLE-RS1 CLE-RS2* genes after rhizobial infection [149, 150]. It is suggested that rhizobia-induced CLE peptides (clavata3/embrio surrounding) or propeptides are transported along the xylem to the shoot, where they are perceived by the LjHAR1/MtSUNN/GmNARK receptors (possibly in combination with other receptors, CLAVATA2 and KLAVIER). [147, 148]. It was also established that LjCLE-RS2 is a post-translational arabinosyl-containing glycopeptide that can directly bind to LjHAR1 receptors [148]. The result of these events may be blocking the formation of nodules.

Creating biotech plants involves a lot of preliminary activities. First of all, the G/E interaction reactions that occur during the key events of ontogeny are subject to analysis. Thus, the features of microspore and microgametogenesis of plants obtained from frozen kernels of winter wheat (*Triticum aestivum* L.) were evaluated [149]. In addition, the passage of mitosis in the roots of germinated plants of the next generation was analyzed with the determination of the energy of germination. The proportion of cells with abnormal mitosis in the roots of seedlings exposed to freezing stress, as well as in control variants, did not exceed 1.5 %. The course of meiosis at the stages of microspore and microgametogenesis in control and experimental variants was also similar in terms of the number of disorders (2 %) and spectra. The authors conclude that such an analysis should be carried out when creating a highly productive, highly adaptive breeding material.

CONCLUSION

Moderm wide-ranging alalysis of “omics”is conducted for wild type genotypes and artificial forms. Among the former plants tolerant and sensitive objectes are tested. For example: to reveal the molecular basis of differential reactions to salt stress in two contrast bean genotypes comparative transcriptome, metabplpme, and ionome there are developed [150, 151, 152]. Transcritomics demonstrated active carbon and amino acid metabolism for the tolerant genotype. Accumulation of lysine, valine, isoleucine in the roots of the sensrtive genotype suggested a halted stress response. Light stress directly inhibits plant physiological reactions. Auxin, gibberelin, ABA increased inder all shadow compared with the control in tissues of *Pinus koraiensis* [153]. The transcription factors MYB-related, AP2-ERF and bHLH specially increased expression during light stress. A total of 911 metabolites were identified and 243 differentially accumulated metabolites (DAMs) were reveald.

Multi-omics investigation is developed with the addition of agricultural plants [151, 154]. Wheat (*Triticum aestivum* L.) can accumulate high contents of heavy metal ions in edible parts. Understanding of wheat response to heavy metal stress and its management in decreasing ions uptake and accumulation may help to improve its growth and grain quality [151]. Rice is one of the most important food crops, feeding more than half of the world’s population. The head milled rice rate (HMRR) is the most important trair of milling quality, which affects the final yield and quality of rice. Transcriptome and metabolome analyses were organized. A total 768 differentially expressed genes (DEGs) were identified between the transcruptome profiles of low-HMRR and high-HMRR accessions. In comparison to the high-HMRR accessions 655 DEGs were up-regulated on the low-HMRR accessions., which was 4.79 folds higher than the nbmber of down-regulated genes [154, 155, 156, 157].

Comparative “omics” analyses can reveal new insights into regulatory mechanisms of entere metabolism.

The study of biotech plants and comparison of various aspects of their metabolism with control parameters showed significant differences. There were also negative aspects along with unconditional successes. It becomes obvious that the guaranteed achievement of the goal is impossible without the involvement of various methods of genetics, molecular biology, and physiology.

A special role can be played by approaches that use the phenomenology of the specificity of the composition of storage proteins, enzymes; features of the accumulation of certain metabolites at various stages of development. In this case, it is possible to isolate mutants with changes in biochemical chains. The study of normal cellular reactions would be appropriate here. Their association with manifestations of economically valuable characteristics can also be a source of a wide array of new information.

New gene alleles can be identified using reliable sequencing technologies. The manifested polymorphism may be coordinated with allelic variants of genes related to the key chains of metabolism.

Information about the molecular structure of a biological system must be coordinated with physiological and biochemical data. It is advisable to support *in vivo* studies with *in vitro* methodologies. The assessment of static structural compartments becomes more objective when combined with an analysis of their dynamic development. Bioinformatic testing of the G/E interaction will provide insights from initial contact and signal perception through signal transduction to response from the body. A combination of methodologies, united by the term “omic tools”, is implemented here. It is possible to create artificial structures with their use. Information transfer systems and approaches will be developed. Genotypes, in which the new genetic material is integrated, already have new improved qualities. Unfortunately, in most cases this new quality has been tested in pot culture. and there are no available field data.

There is a constant search for new genes (groups of genes) that are coordinated with tolerance. The discovery of new genes in the plant genome is expected, followed by determination of their exact physiological role in stress tolerance using functional genomics.

However, the achieved successful results form new tasks, create significant risks associated with both the creation and use of genetically modified organisms. To overcome these and new (unexpected) hurdles, a continuous comprehensive study of the functional characteristics of new genotypes under normal conditions, stresses *in vivo* and *in vitro*, and also under

varying growing conditions is required. This will highlight the multilevel cross-links in the plant body. This approach seems to be very promising, as it is coordinated by changes in the habitat.

At the same time, it should be noted that genetic engineering should not be considered as a universal biotechnology. Undoubtedly, it has its limitations, like any other. But this circumstance should stimulate the conduct of new research, the creation of related areas of research, and the development of new hypotheses. The results of the research make it possible to raise the question of new directions - metabolic engineering and metabolic (associated with the activity of individual enzymes) cell selection. In our opinion, the most effective approach will be a combination of competitiveness of ideas and a combination of research methods. At the same time, any new direction should be closely linked to important biological and ethical postulates. In this case, it is necessary to properly build a chain of priorities.

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