

Perspective of ...omics research development in kazakhstan as a new step of plant biotechnology in post- genomics era

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Abstract. The next generation of biotech crops promises to include a broad range of products that will provide benefits to both farmers and consumers, and continue to meet the global agricultural challenges. These products will most likely involve regulation of key endogenous plant pathways resulting in improved quantitative traits such as yield, photosynthesis, biotic and abiotic stress tolerance.

The emergence of the novel 'omics' technologies, such as genomics, proteomics and metabolomics, is now allowing researchers to identify the genetic behind plant stress responses.

To date, successes in genetic improvement of environmental stress resistance have included manipulation of a single or a few genes involved in signaling/regulatory pathways or that encode enzymes involved in these pathways. In recent years, various excellent reviews summarized the current knowledge on structural genes involved in phenylpropanoid, specifically lignin and flavonoid formation, regulatory transcription factors, hormonal control of the whole pathways by jasmonate or auxin and evolution of pathway genes from primary metabolism. Genetic engineering of the key metabolic pathways is a powerful tool for crop improvement in new step Biotech in Post-Genomics era.

Soybean diseases world-wide is one of the serious problems that reduce yield up to 11%-30% of the total production. In many countries disease-control in soybean is limited only by agricultural technologies.

The main idea of our research is to improve soybean innate resistance to biotic stresses via genetic engineering of the phenylpropanoid pathway, namely – introduction into soybean key genes involved in lignin biosynthesis, - the compound that is assigned to a broad range of physiological processes participating in plant growth, providing the rigidity to the cell walls, the natural mechanical barrier and defense against pathogen penetration. Proposed approach to soybean diseases combat is included method of molecular cloning and constructing of transcriptional factor *PTMyb*, key genes involved in lignin biosynthesis: *PAL*, *C4H/F5H*, *CAD*, *COMT*, etc., followed by genes identification and sequencing in collaboration with UIUC, USA; optimization of germ-line genetic transformation technology; screening and molecular detection of transgenes by PCR and RT-PCR analysis; analysis of physiological and biochemical consequences of these valuable genes introduction into soybean; analysis of lignin biosynthesis parameters and metabolic profiling of transgenic plants; analysis of transgenes to micro-pathogens resistance; methods of phenology, morphology, productivity characterization.

Obtained Results: 1. Gene constructs of key genes involved in lignin biosynthesis, prepared for introduction into soybean. 2. Optimized germ-line genetic transformation technique for soybean transformation. 3. Molecular confirmed soybean transgenes of T₁ - T₂ generations with valuable genes. 4. Biochemical confirmation of increased lignin biosynthesis, metabolic profiling. So, transition is achieved from Genome to Phenome in post – Genomics era.

Introduction

Actuality. The main idea of our research is to review perspective of ...omics research development world-wide and in Kazakhstan as a new step of plant biotechnology in post-genomics era, and to improve soybean innate resistance to stresses and thus – yield, via genetic engineering of the key metabolic pathways.

The next generation of biotech crops promises to include a broad range of products that will provide benefits to both farmers and consumers, and continue to meet the global agricultural challenges. These products will most likely involve regulation of key endogenous plant pathways resulting in improved quantitative traits such as yield, photosynthesis, biotic and abiotic stress tolerance. Genetic engineering of the key metabolic pathways is a powerful tool for crop improvement in new step Biotech in Post-Genomics era [1,2].

To date, successes in genetic improvement of environmental stress resistance have involved manipulation of a single or a few genes involved in signaling/regulatory pathways or that encode enzymes involved in these pathways. The emergence of the novel ‘omics’ technologies, such as genomics, proteomics and metabolomics, is now allowing researchers to identify the genetic behind plant stress responses [3].

Improvement of soybean innate resistance to biotic stress via genetic engineering of the phenylpropanoid metabolic pathways – one of the key metabolic pathway of secondary metabolism, seems to be very promising for combat with soybean diseases. The general phenylpropanoid metabolism generates an enormous array of secondary metabolites based on the few intermediates of the shikimate pathway as the core unit. In recent years, various excellent reviews summarized the current knowledge on structural genes involved in phenylpropanoid, specifically lignin and flavonoid formation, regulatory transcription factors, hormonal control of the whole pathways by jasmonate or auxin and evolution of pathway genes from primary metabolism [4-6]. Improvement of soybean innate resistance to stresses via genetic engineering of phenylpropanoid pathway namely include introduction into soybean key genes involved in lignin biosynthesis, - the compound that is assigned to a broad range of physiological processes participating in plant growth, providing the rigidity to the cell walls, the natural mechanical barrier and defense against pathogen penetration [7-8].

1 Soybean diseases world-wide and in Kazakhstan is one of the serious problems that reduce its yield up to 11-30%, but they have been studied not enough [9]. In many countries disease-control in soybean is limited only by agricultural technologies [10]. Everywhere prevalent diseases caused by microorganisms and micro-fungus such as downy mildew, pathogen - microfungus *Peronospora manshurica* (Naum.); brown leaf spot, caused by microfungi *Phyllosticta sojaecola* Mass, *Phytophthora*, but disease-control in soybean limited only by agricultural technologies. Important for Kazakhstan's goal is to create resistant to biotic stresses - diseases caused micro- pathogens, highly productive soybeans via genetic engineering. Important for Kazakhstan's goal is to create resistant to biotic stresses - diseases caused micro- pathogens, highly productive soybeans via genetic engineering. World-wide losses due to diseases are estimated at 11% of the total production [11, 12, 13]. Plant resistance is an economical and sustainable disease management option. Efforts to increase the strength of the innate defense system like lignin biosynthesis would help limit colonization of these pathogens [14]. Genetic engineering of the key metabolic pathways components with a broad range of products, including improvement of complex plant resistance to stress and yield increasing.

2 **Objective:** To establish approaches to improve soybean innate resistance to biotic stresses and to create resistant soybean via genetic engineering of the phenylpropanoid pathway for increasing biosynthesis of lignin – natural anti-microbial compounds, in order to improve management of microorganisms caused diseases.

Methodology. Proposed approach to soybean diseases combat is included method of molecular cloning and constructing of transcriptional factor *PTMyb*, key genes involved in lignin biosynthesis: *PAL*, *C4H/F5H*, *CAD*, *COMT*, followed by genes identification and sequencing in collaboration with UIUC, USA; optimization of germ-line genetic transformation technology; screening and molecular detection of transgenes by PCR and RT-PCR analysis; analysis of physiological and biochemical consequences of these valuable genes introduction into soybean; analysis of lignin biosynthesis parameters and metabolic profiling of transgenic plants; analysis of transgenes to micro-pathogens resistance; methods of phenology, morphology, productivity characterization.

Obtained results: 1. Gene constructs of key genes involved in lignin biosynthesis – *PAL* (phenylalanine ammonia lyase), *CCR* (cinnamoyl CoA reductase), *ac*-gene – encoded chitin binding proteins – anti-microfungus, *FeSOD* gene – (Fe-dependent superoxide dismutase – anti-ROS), prepared for introduction into soybean. 2. Optimised genetic transformation technique for soybean transformation

and introduction of valuable genes into soybean. 3. Molecular confirmed soybean transgenes with valuable genes. Soybean transgenic seeds of T₁-T₂, resistant to diseases caused by microbial penetration - biotic stresses. 4. Biochemical confirmation of increased lignin biosynthesis. Phenotypic, morphological, and productivity consequences of soybean genetic engineering to stress resistance.

Creation and confirmation of the transgenic soybean plants resistance to biotic stresses include biotechnological, molecular methods and global metabolic profiling, so transition is achieved from Genome to Phenome in post – Genomics era. The ultimate goal of this work is to produce new strains of soybean for breeders and biodiversity with improved biotic and abiotic stress resistance is achieved.

Review of ...omics research

3 Agricultural crops fulfilling future food and fuel needs must display both high intrinsic yield and yield stability under biotic and abiotic stresses. Annual increases in yield achieved from traditional breeding programs worldwide are no longer sufficient to meet projected demand for major cereal crops: rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*) and soybean (*Glycine max*, L.) [15]. With the burgeoning world population, cereal grain yields alone must increase by at least 70% before 2050 [16].

4 World-wide losses due to diseases are estimated at 11% of the total production. Plant resistance is an economical and sustainable disease management option. Efforts to increase the strength of the innate defense system like lignin biosynthesis would help limit colonization of these pathogens. Genetic engineering of the key metabolic pathways components with a broad range of products, including improvement of complex plant resistance to stress and yield increasing.

5 Biotechnological approaches to study plant responses to stress at present are: Multiple biotic and abiotic environmental stress factors affect negatively various aspects of plant growth, development and crop productivity. Plants, as sessile organisms, have developed, in the course of their evolution, efficient strategies of response to avoid, tolerate or adapt to different types of stress situations. Over the last few decades advances in plant physiology, genetics, and molecular biology have greatly improved our understanding of plant responses to abiotic stress conditions. Recent progresses on systematic analyses of plant responses to stress including genomics, proteomics, metabolomics, and transgenic-based approaches are summarized [2]. The emergence of the novel ‘omics’ technologies, such as genomics, proteomics and metabolomics, is now allowing researchers to identify the genetic behind plant stress responses [3].

“The English-language neologism omics informally refers to a field of study in biology ending in -omics, such as genomics, proteomics or metabolomics. The related suffix -ome is used to address the objects of study of such fields, such as the genome, proteome or metabolome respectively” [17]. Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. The suffix -ome as used in molecular biology refers to a *totality* of some sort; it is an example of a "neo-suffix" formed by abstraction from various Greek terms in -ωμα, a sequence that does not form an identifiable suffix in Greek.

The following definition of ‘omics’ can be found on The Omics Wikipedia:

"Omics is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in various ‘omes’. The main focus is on: 1) mapping information objects such as genes, proteins, and ligands; 2) finding interaction relationships among the objects; 3) engineering the networks and objects to understand and manipulate the regulatory mechanisms; and 4) integrating various omes and omics subfields”.

The Oxford English Dictionary (OED) distinguishes three different fields of application for the -ome suffix:

1. in medicine, forming nouns with the sense "swelling, tumour";
2. in botany or zoology, forming nouns in the sense "a part of an animal or plant with a specified structure";
3. in cellular and molecular biology, forming nouns with the sense "all constituents considered collectively".

The -ome suffix originated as a variant of -oma, and became productive in the last quarter of the 19th century. It originally appeared in terms like sclerome or rhizome. All of these terms derive from Greek

words in -ωμα, a sequence that is not a single suffix, but analyzable as -ω-μα, the -ω- belonging to the word stem (usually a verb) and the -μα being a genuine Greek suffix forming abstract nouns.

The OED suggests that its third definition originated as a back-formation from *mitome*. Early attestations include *biome* (1916) and *genome* (first coined as German *Genom* in 1920).

The association with *chromosome* in molecular biology is by false etymology. The word *chromosome* derives from the Greek stems χρωμ (ατ)- "colour" and σωμα (ατ) - "body". While σωμα "body" genuinely contains the -μα suffix, the preceding -ω- is not a stem-forming suffix but part of the word's root. Because *genome* refers to the *complete* genetic makeup of an organism, a neo-suffix *-ome* suggested itself as referring to "wholeness" or "completion".

Bioinformaticians and molecular biologists figured amongst the first scientists to apply the "-ome" suffix widely. Early advocates included bioinformaticians in Cambridge, UK, where there were many early bioinformatics labs such as the MRC centre, Sanger centre, and EBI (European Bioinformatics Institute). For example, the MRC centre carried out the first genome and proteome projects.

Omics is a new research field in which all the biological data obtained from various studies including genomics, transcriptomics and proteomics are comprehensively collected, integrated and analyzed to decipher the biological nature of living organisms (figure 1). Today's marvelous innovation in DNA sequencing technology has enabled us to notably accelerate the accumulation of and analysis of various biological data. This has resulted in a shift of Omics research to the next generation in which the amount of biological data to be handled will be increased by two or more orders of magnitude than that analyzed to date. New datasets will also be produced from emerging research fields such as epigenomics and metagenomics.

Kinds of omics studies: Genomics, Proteomics, Metabolomics, Phenomics.

Genomics – study of the genomes of organisms (figure 2).

The genome is a store of biological information but on its own it is unable to release that information to the cell. Utilization of the biological information contained in the genome requires the coordinated activity of enzymes and other proteins, which participate in a complex series of biochemical reactions referred to as genome expression. The initial product of genome expression is the **transcriptome**, a collection of RNA molecules derived from those protein-coding genes whose biological information is required by the cell at a particular time. The transcriptome is maintained by the process called **transcription**, in which individual genes are copied into RNA molecules. The second product of genome expression is the **proteome**, the cell's repertoire of proteins, which specifies the nature of the biochemical reactions that the cell is able to carry out. The proteins that make up the proteome are synthesized by translation of the individual RNA molecules present in the transcriptome.

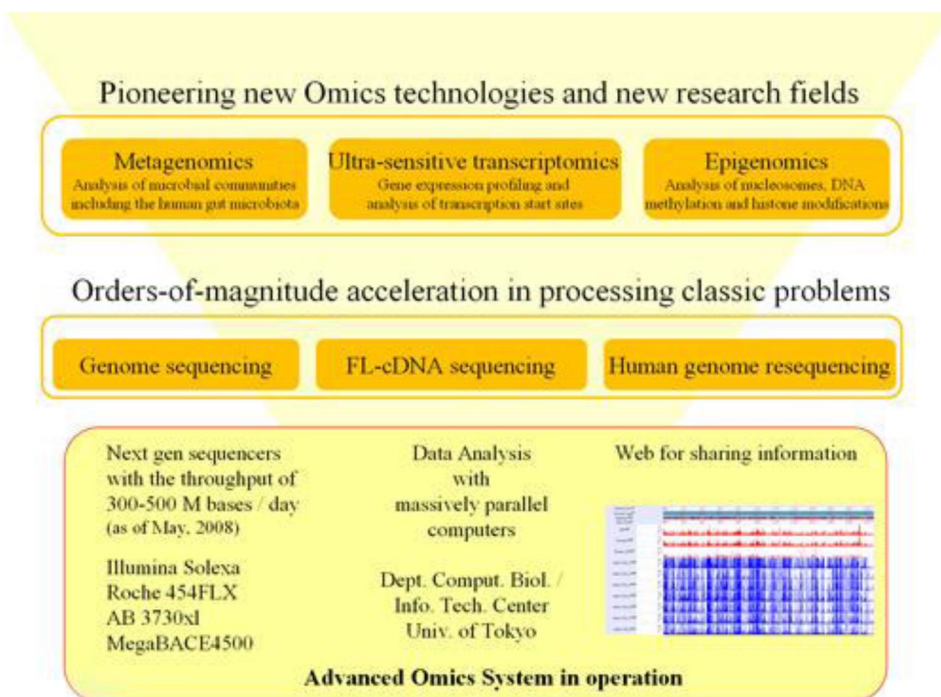


Figure 1. Pioneering new Omics technologies and new research fields

Modern information about genomes and genome expression includes explanations how genomes are studied, how they are organized, how they function, and how they replicate and evolve. These knowledge was not possible until very recently. Since the 1950s, molecular biologists have studied individual genes or small groups of genes, and from these studies have built up a wealth of knowledge about how genes work. But only during the last 10 years have techniques been available that make it possible to examine entire genomes. Individual genes are still intensively studied, but information about individual genes is now interpreted within the context of the genome as a whole. This new, broader emphasis applies not just to genomes but to all of biochemistry and cell biology. No longer is it sufficient to understand individual biochemical pathways or subcellular processes. The challenge now is provided by **systems biology**, which attempts to link together these pathways and processes into networks that describe the overall functioning of living cells and living organisms. Knowledge of genomes show how this exciting area of research is underpinning our developing understanding of biological systems.

Firstly must be paid attention to the basic principles of molecular biology by reviewing the key features of the three types of biological molecule involved in genomes and genome expression: **DNA**, **RNA**, and **protein** [18].

Genomics includes several directions:

Cognitive genomics examines the changes in cognitive processes associated with genetic profiles.

Comparative genomics: Study of the relationship of genome structure and function across different biological species or strains.

Functional genomics: Describes gene and protein functions and interactions (uses microarray kind of techniques). Functional genomics aims at identifying the functions of as many genes as possible of a given organism. It combines different -omics techniques such as **transcriptomics** and **proteomics** with saturated mutant collections.

Metagenomics: Study of metagenomes, i.e., genetic material recovered directly from environmental samples.

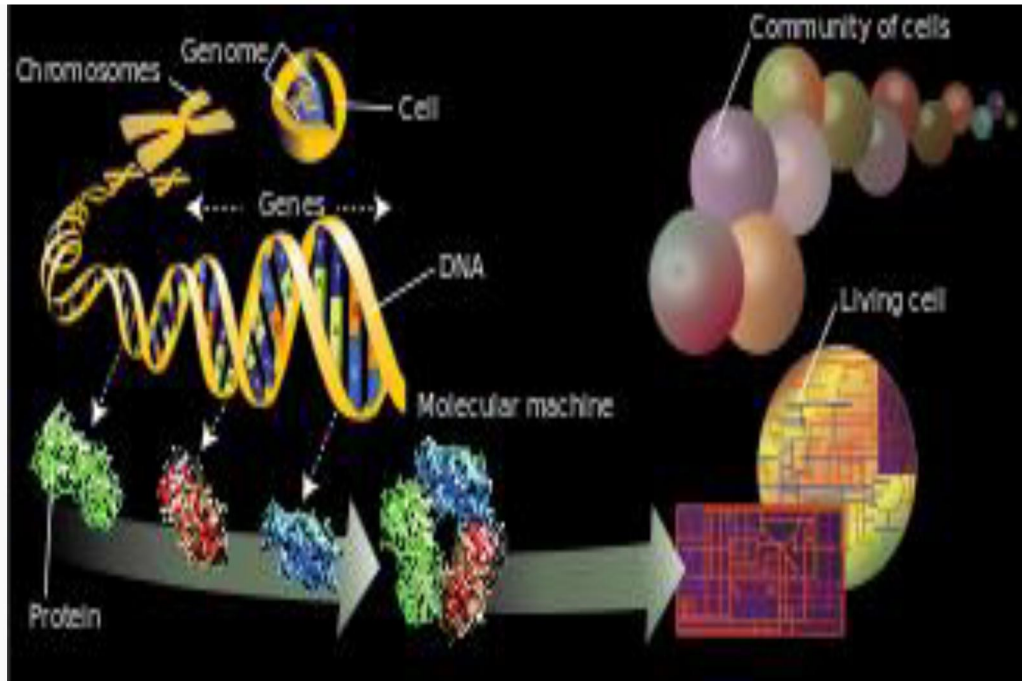


Figure 2. Diagram illustrating genomics

Personal genomics: Branch of genomics concerned with the sequencing and analysis of the genome of an individual. Once the genotypes are known, the individual's genotype can be compared with the published literature to determine likelihood of trait expression and disease risk. Helps in Personalized Medicine.

Epigenomics: Study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome. ChIP-Chip and ChIP-Seq technologies used.

Lipidomics: Lipidome is the entire complement of cellular lipids, including the modifications made to a particular set of lipids, produced by an organism or system. Lipidomics includes large-scale study of pathways and networks of lipids. Mass spectrometry techniques are used.

Proteomics. What Is Proteomics? [19].

A bacterial cell may seem simple but it's actually a complex structure — a gel-like matrix of the cytoplasm, surrounded by both a lipid bilayer cell membrane and a cell wall. The cell must perform many functions including the intake of nutrients, the metabolism of those nutrients, growth, cell division, and the excretion of wastes. What molecules are involved? Although the cytoplasm contains water, proteins, carbohydrates, various ions and assorted other molecules, proteins do most of the work. A typical bacterium requires more than 4,000 proteins for growth and reproduction. Not all of the proteins are made at the same time and some are made only under special conditions, such as when the cell is stressed or finds itself in a novel environment. Proteomics - large-scale study of proteins, particularly their structures and functions. Mass spectrometry techniques are used.

The complement of proteins found in this single cell in a particular environment is the **proteome**. Proteomics is the study of the composition, structure, function, and interactions of the proteins directing the activities of each living cell [20].

Kinds of proteomics are:

Immunoproteomics: study of large sets of proteins (proteomics) involved in the immune response.

Nutriproteomics: Identifying the molecular targets of nutritive and non-nutritive components of the diet. Uses proteomics mass spectrometry data for protein expression studies.

Proteogenomics: An emerging field of biological research at the intersection of proteomics and genomics. Proteomics data used for gene annotations.

Structural genomics: Study of 3-dimensional structure of every protein encoded by a given genome using a combination of experimental and modeling approaches.

Transcriptomics. Transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA,

and other non-coding RNA, produced in one or a population of cells.

Metabolism study:

Metabolomics: Scientific study of chemical processes involving metabolites. It is a "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles. **Metabolome** - is the complete complement of small molecules present in an organism. Metabolomics is helping us to bring these diverse biochemical differences better into view.

Metabonomics: The quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification. Metabonomics is a non-plant term generally used to define the technology used to measure quantitatively the metabolic composition of body fluids.

Metabolomics provides the possibility of monitoring a complete set of metabolites what could largely improve the understanding of many physiological plant processes [21]. This field of "metabolomics", while still in its infancy, has nevertheless already been welcomed with open arms by the plant science community, partly because of these said advantages but also because of the broad potential applicability of the approaches in both fundamental and applied science.

Metabolomics is the technology which has been developed to assist in the biochemical analysis of complex mixtures. The ultimate aim is to have a technology which permits essentially unbiased, quantitative biochemical analysis of all the components in an extract of a biological material. Key to this is having a functional combination of comprehensiveness, analytical precision, and sample throughput. While full metabolite quantification is perhaps a Holy Grail, in many cases, semi-quantification or even relative values of one sample to another may be sufficient. In plants, the challenge is particularly daunting, as plants are renowned for the diversity of the chemicals they can produce and the complexity of the individual molecules involved. The chemical composition of plant tissues is also highly dependent both on internal (genetic) factors as well as external (environmental) factors, all of which must also be placed within the concept of tissue differences, where even adjacent cell layers may contain highly contrasting biochemical profiles [22].

Metabolomics is the technology geared towards providing an essentially unbiased, comprehensive qualitative and quantitative overview of the metabolites present in an organism. This technological tool, recently developed, includes different approaches, namely **targeted analysis**, **metabolic fingerprinting** and **metabolite profiling** [23]. It is used to measure the concentration of a limited number of known metabolites precisely, by using either **gas chromatography (GC)** or **liquid chromatography (LC)** coupled to **mass spectrometry (MS)** or **nuclear magnetic resonance spectroscopy (NMR)**.

Some useful working definitions (figure 3):

Metabolic fingerprinting. High-throughput qualitative screening of the metabolic composition of an organism or tissue with the primary aim of sample comparison and discrimination analysis. Generally no attempt is initially made to identify the metabolites present. All steps from sample preparation, separation, and detection should be rapid and as simple as is feasible. Often used as a forerunner to metabolic profiling.

Metabolic profiling – is identification and quantification of the metabolites present in an organism. For practical reasons this is generally only feasible for a limited number of components which are generally chosen on the basis of discriminant analysis or on molecular relationships based upon molecular pathways or networks.

Targeted analysis – is following broad-scale metabolomics analysis, or based upon prior knowledge, biochemical profiling can be performed in greater detail on selected groups of metabolites by using optimized extraction and dedicated separation/detection techniques. Target analysis is constrained to one or a very few target compounds (such as hormones). Such targets are usually quantified in an absolute manner using calibration curves and/or stable isotope-labeled internal standards.

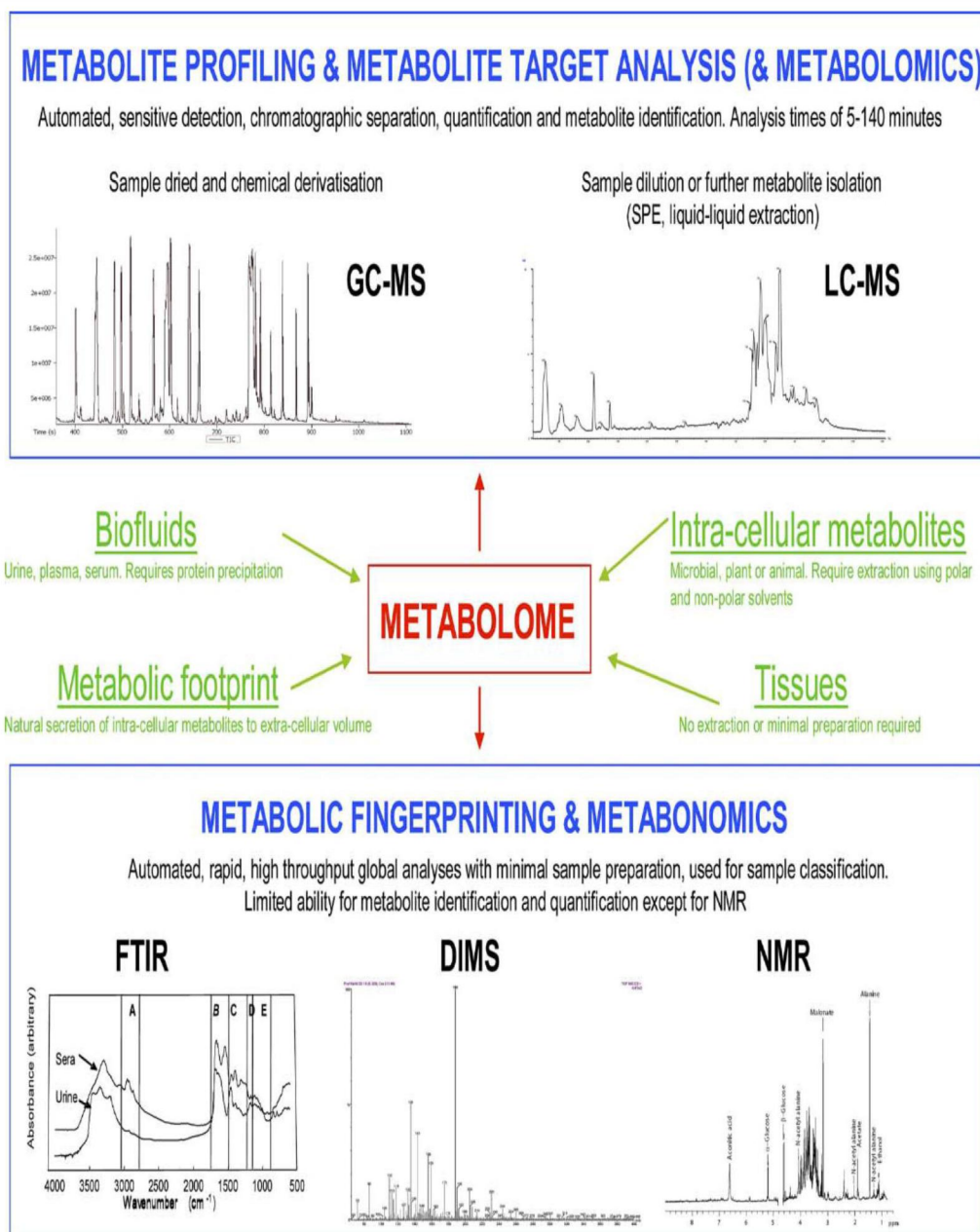


Figure 3. Summary of the different metabolomics-based strategies for sample preparation and sample analysis [24]

The exact application depends on the crop, but many crops have already been subjected to a metabolomics assessment including major food crops such as rice, wheat, tomato, melon, *Brassica*, coffee, and potato. Such knowledge is highly complementary to that obtained from the more traditional and established methods and as such, metabolomics will likely provide additional tools to help advance plant breeding strategies and the speed of developing new varieties more suited to current demands.

Plant metabolomics is a field of science which is still in a dynamic phase of development. Perhaps the achievements already booked in terms of analytical capacity, precision, and throughput raise even more new questions than have answered old ones. Nevertheless, the potential has clearly been demonstrated and examples of good practice are presented here. Techniques and equipment for both chemical and data analysis improve constantly, but robust procedures for their application will clearly always be required [25].

Metabolite profiling has been established as a multiparallel strategy for relative quantification of a mixture of compounds or compound classes using chromatography and universal detection technologies

(gas chromatography–mass spectrometry [GC–MS], liquid chromatography–MS). Despite its origins dating back to the late 1960s, it was only in the 1980s that its use was acknowledged to diagnose metabolic disorders in men, especially for rapid screening of inborn errors. Even faster electrospray ionization–MS/MS screening methods replaced longish chromatographic methods, and method development had stopped despite its potential use for other, less imminent diseases such as likelihood assessments of type II diabetes mellitus or cardiovascular risk factor evaluation. In addition to its diagnostic use, profiling blood samples can be employed to investigate specific biochemical responses. The broader scope of analysis outweighs the disadvantages by taking compromises in method development and the reduced accuracy for specific metabolites.

A major difference to profiling plant tissues is that no fractionation step is utilized, enabling the analysis of primary metabolites like sugars and amino acids concomitant with lipids such as sterols and free fatty acids. Metabolite profiling is an analytical method for relative quantification of a selected number of metabolites from biological samples, i.e., members of specific pathways or compound classes. Metabolite profiling is distinguished from other analytical procedures by its scope: Metabolite profiling restricts itself to a certain range of compounds or even to screening a predefined number of members of a compound class. Within these constraints, a single analytical platform may be sufficient. Examples might be the analysis of carotenoid intermediates by high-performance liquid chromatography/diode array ultraviolet detection (HPLC–UV), or sugars, hydroxy acids, and amino acids by fractionation and gas chromatography–mass spectrometry (GC–MS), or vitamin profiling by HPLC–MS/MS. Quantification in metabolite profiling is usually carried out relative to comparator samples, such as positive and negative controls.

Metabolite profiling, therefore, must be seen as a compromise between truly quantitative target analysis and completely unbiased metabolomics. Each metabolite profiling method is directed toward a chemically different compound class, hence, there are various methods published depending on the actual task. In itself, each procedure will be a compromise between several parameters, such as compound stability, solubility, influence of the cellular matrix, time needed to carry out the protocol, constraints given to garner samples (blood withdrawal), extraction (potentially followed by fractionation), submission to analytical instruments, raw data analysis, and statistics.

Validation criteria for metabolite profiling and metabolomics protocols are, therefore, different from target analysis: (1) reproducibility (precision of relative metabolite levels) is more important than absolute recovery. (2) Robustness and practicability are more important than accuracy (correctness in absolute metabolite concentrations). (3) Comprehensiveness is more important than inclusion of a certain metabolite that might be missed. (4) Overall dynamic range for the majority of compounds is more important than the detection limit for a specific substance. (5) On the contrary, the ability to include important known key metabolites may still be more important than the detection of unidentified peaks that might be biochemical side products of enzymes with low substrate specificity [23].

Methods such as mRNA expression profiling have provided a vast amount of genomic and transcriptomic information about plants and other organisms. However, there is explicit indication that considerable metabolic control is executed on the metabolite and on the protein level including protein modifications, thereby constituting the phenotypic plasticity. Consequently, the analysis of the molecular phenotype demands the step toward mass spectrometry (MS)-based postgenomic techniques such as metabolomics and proteomics [26–27].

Phenomics. A key goal of biology is to understand phenotypic characteristics, such as health, disease and evolutionary fitness. Phenotypic variation is produced through a complex web of interactions between genotype and environment, and such a ‘genotype–phenotype’ map is inaccessible without the detailed phenotypic data that allow these interactions to be studied. Despite this need, our ability to characterize phenomes — the full set of phenotypes of an individual — lags behind our ability to characterize genomes. Phenomics should be recognized and pursued as an independent discipline to enable the development and adoption of high-throughput and high-dimensional phenotyping [16]. Plant phenomics - plant physiology in an ‘omics’ perspective, review show some of the new highthroughput and high-resolution phenotyping tools and discuss their application to plant biology, functional genomics and crop breeding. Plant biology faces new challenges: a role for plant phenomics. Global agriculture and the plant biology underpinning it are facing major challenges which require new approaches to functional genomics

and plant breeding. Global food security, the identification of appropriate and efficient plant-based biofuel feedstocks and coping with climate change are foremost in the minds of scientists, politicians and the general public. To address these issues, we need new high-yielding genotypes of agricultural crops adapted to our future climate.

High-throughput phenomics of model systems: the phenomics–genomics pipeline. A clear goal of phenomics is to bridge the gap between genomics, plant function and agricultural traits. Particularly in the context of model systems, where availability of genomic sequence is burgeoning, there is a pressing need for a searchable phenotypic database linking gene sequence to plant structure, development, composition and performance, all measured in a clearly defined environment. Recent advances in DNA sequencing and phenotyping technologies, in concert with analysis of large datasets have spawned '**phenomics**', **the use of large scale approaches to study how genetic instructions from a single gene or the whole genome translate into the full set of phenotypic traits of an organism.** Phenotyping is frequently slower and more expensive than genomics due to the difficulties of measuring molecular, cellular, or organismal traits with sufficient throughput, resolution, and precision. Phenomics can be used across the full range of biological sciences - from studies of monocultures in well-defined and controlled laboratory environments through agricultural field conditions to populations of organisms under rapidly changing conditions. Thus, phenomics has broad importance in applied and basic biology and is equally relevant to goals as disparate as yield improvement in food and energy crops, environmental remediation using microbes and plants and understanding complex networks that control fundamental life processes.

Phenome. How is phenome different from phenotype?

Phenomics, the study of the phenome, is a rapidly emerging area of science, which seeks to characterize phenotypes in a rigorous and formal way, and link these traits to the associated genes and gene variants (alleles). Examples of phenotypic parameters include gross morphological measures such as cell size, tree height or crops yield, dynamic measures such as rate of cell division of a unicellular organism, metabolism or nutrient uptake, and molecular measures such as mass spectrometry fingerprints and transcript profiles.

Formally, phenomics is the science of large-scale phenotypic data collection and analysis, whereas **the phenome is the actual catalog of measurements.** While it shares characteristics with classical mutant screening or quantitative trait analysis, it is distinguished from these traditional approaches in scale and scope.

First, phenomic studies typically employ large populations of genetic variants with the goal of sampling variation in many or all genes. Second, each genotype is assayed for a large number of traits, typically using well-tested and high-throughput standard operating procedures with systems in place to maximize accuracy in sample tracking and data reproducibility. Third, key features of the growth conditions are well defined and closely monitored. Finally, the phenotypic data and metadata descriptions of the experimental conditions are captured in formats that allow detailed data analysis. These analyses would ideally identify relationships between genotype and phenotype as well as reveal correlations between seemingly unrelated phenotypes (Schauer et al., 2006; Lu et al., 2008) or genetic loci (Gerke et al., 2009).

Because most phenotypes are determined by the interactions of genes and environment, the ideal situation is to collect large numbers of measures across multiple environments, at different developmental stages, and for multiple cell/tissue/organ types.

Imaging. Imaging is ideal for phenomic studies owing to the availability of many technologies that span molecular to organismal spatial scales, the intensive nature of the characterization and the applicability of generic segmentation techniques to data. Spatial or temporal data on many phenotype classes such as morphology, behaviour, physiological state, and locations of proteins and metabolites can be captured in intensive detail by imaging. Spectroscopic imaging of crop plants can be used to predict many properties on very large populations.

Functional genomics. Global soybean production is frequently impacted by various stresses, including both abiotic and biotic stresses. To develop soybean plants with enhanced tolerance to different stressors, functional genomics of soybean and a comprehensive understanding of available biotechnological resources and approaches are essential. Recent advances in soybean functional genomics provide unprecedented opportunities to understand global patterns of gene expression, gene regulatory

networks, various physiological, biochemical, and metabolic pathways as well as their association with the development of specific phenotypes. Soybean functional genomics, therefore, will ultimately enable us to develop new soybean varieties with improved productivity under adverse conditions by genetic engineering [28].

Plant functional genomics, therefore, has emerged as an alternative and rapidly evolving scientific discipline for studies of the functions of genes and genetic engineering of plants aimed at improving plant productivity in adverse environments. In the last decade, considerable progress has been made in developing various resources and tools, including the entire soybean genomic sequence, full-length cDNA (FL-cDNA) collections, mutant and germplasm resources, molecular markers and “-omics” tools for soybean functional genomics, which in turn provide an effective way for genetic modification of economically important crops by gene transfer. Already, the extensive body of soybean sequence data has facilitated cloning of genes of interest and given better understanding into soybean evolution. Integration of genetic and genomic data from multiple legume and plant species also provides support for soybean genome annotation and comparative functional genomics. Plant biology has been and will continue to be revolutionized by functional genomics researches [29-31].

The soybean (*Glycine max* L.) is one of the priorities food and feed crops in Kazakhstan, USA and world-wide. Soy – is widely used food product, thanks to the high content of vegetable protein, an average of about 40-50% by weight of the seed, much like animal protein, and a relatively high yield. Soybean is used as substitutes for animal products. Currently, soy is one of the major crops in Kazakhstan, and the creation of new high-yielding, resistant to biotic stresses (pests and diseases) forms of this culture by genetic engineering is prospective and promises high socio-economic and environmental effects.

Soybean is one of the major legume crops native to East Asia. Research on soybean is driven by its importance as a food crop worldwide. Soybean presents a wealth of resources for utilization, including proteins, oils, mineral nutrients, and natural products such as isoflavonoids that impact human health and nutrition. Its products are widely used as a protein source and edible vegetable oil for human consumption, and high-protein feed supplements for the chicken and pork industries [32-33].

Soybean diseases world-wide and in Kazakhstan is one of the serious problems that reduce its yield up to 11-30%, but they have been studied not enough. In many countries disease-control in soybean is limited only by agricultural technologies. Everywhere prevalent diseases caused by micropatogenes and micro-fungul such as downy mildew, pathogen - microfungul *Peronospora manshurica* (Naum.); brown leaf spot, caused by microfungi *Phyllosticta sojaecola* Mass, *Phytophthora*, but disease-control in soybean limited only by agricultural technologies. Important for Kazakhstan's goal is to create resistant to biotic stresses - diseases caused micro- pathogens, highly productive soybeans via genetic engineering. Important for Kazakhstan's goal is to create resistant to biotic stresses - diseases caused micro- pathogens, highly productive soybeans via genetic engineering. World-wide losses due to diseases are estimated at 11% of the total production. Plant resistance is an economical and sustainable disease management option. Efforts to increase the strength of the innate defense system like lignin biosynthesis would help limit colonization of these pathogens. Genetic engineering of the key metabolic pathways components with a broad range of products, including improvement of complex plant resistance to stress and yield increasing. Soybean growth, productivity and seed quality are adversely affected by a wide range of stresses, including both abiotic and biotic stresses [32, 34, 35].

As for biotic stresses, soybean cyst nematode (SCN), caused by *Heterodera glycines*, is the most widespread and damaging chronic disease of soybean worldwide [36]. SCN infestations can be controlled to an extent by crop rotation and tillage practices, but once established, the nematode population cannot be completely eliminated by these methods. Planting SCN resistant cultivars is the most effective and efficient means of control. In addition, soybean rust (SBR), caused by *Phakopsora pachyrhizi* and *P. meibomia*, has been considered one of the major diseases in Asia and South America for many years and more recently in the US [37].

Root and stem rot disease caused by *Phytophthora sojae* is rapidly becoming a very destructive soybean disease in the US as well [38]. Soybean mosaic virus (SMV) is also noteworthy. The SMV disease, which is spread by aphids during growing season, has caused the most yield losses in some soybean growing countries in Asia such as China, Indonesia, and Korea [39, 40]. Facing with various

stressors, breeders take a relatively traditional approach. They grow and cross varieties, then evaluate how the progenies vary in their ability to deal with stresses. The best-adapted plants will be then selected for growing in fields exposed to stresses.

Since commercialization of the first GM soybean in 1996, farmers have planted more than 690 million ha (1.7 billion acres) [11, 12]. The first generation of biotech crops focused primarily on the single gene traits of herbicide tolerance and insect resistance. The next generation of biotech crops promises to include a broad range of products that will provide benefits to both farmers and consumers, and continue to meet the global agricultural challenges. These products will most likely involve regulation of **key endogenous plant pathways** resulting in improved quantitative traits such as yield, photosynthesis, biotic and abiotic stress tolerance [1].

Soybean is **one of major producers of beneficial secondary compounds like phenols and lignin**, which possess health-promoting properties, thereby enjoy popular use in industrial and pharmaceutical applications [41]. Recently, soybean has also emerged as a resource for production of biodiesel [42]. The largest producer of soybean is USA with 70.4 million metric tons. Other major countries such as Brazil, Argentina, and China contributed 61, 47 and 14.3 million metric tons, respectively [43]. Soybean is one of priority crop in Kazakhstan.

Biotechnologists, meanwhile, have taken advantage of recent advances in functional genomics and biotechnology to **genetically engineer crops** which can give better yield than the unmodified ones in adverse conditions [32, 35, 44, 45]. Although traditional methods of plant breeding have made a significant contribution to soybean improvement, but progress has been slow in targeting complex traits like abiotic and biotic resistances [28, 35]. We have good experience to elaborate and patent new simple and effective technique for soybean genetic transformation for abiotic stress resistance [46, 47] and now conduct researchers for soybean genetic engineering to improve lignin biosynthesis as natural barrier against micropathogen penetration caused number of diseases.

Omics technology for functional genomics of soybean. Transcriptomics. The availability of large data set of ESTs has led to the development of cDNA and oligo microarray platforms for transcriptomics in soybean [48]. Metabolomics is the apogee of the omics trilogy. Metabolites, the chemical entities that are transformed during metabolism, provide a functional readout of cellular biochemistry. With emerging technologies in mass spectrometry, thousands of metabolites can now be quantitatively measured from minimal amounts of biological material, which has thereby enabled systems-level analyses. By performing global **metabolite profiling**, also known as untargeted metabolomics, new discoveries linking cellular pathways to biological mechanism are being revealed and are shaping our understanding of cell biology, physiology and medicine (figure 4).

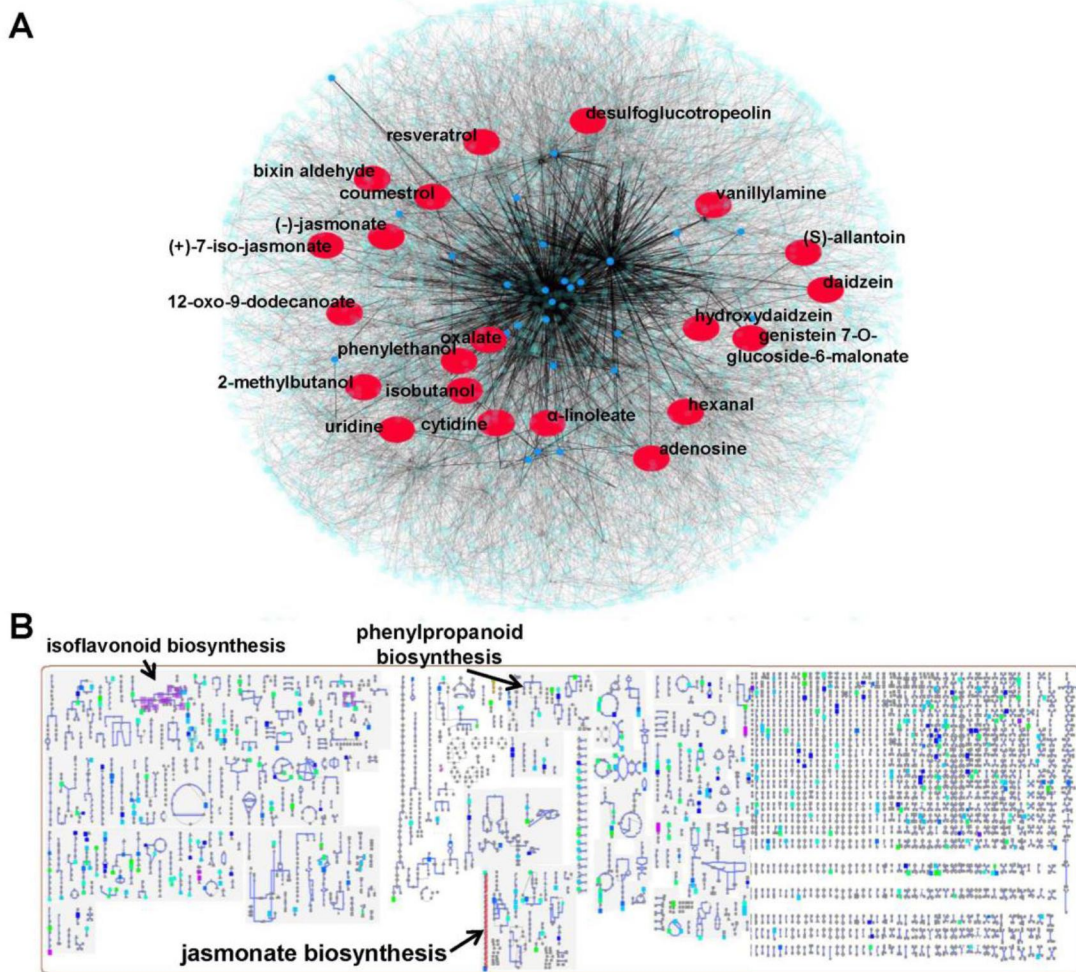


Figure 4. Global soybean metabolome and its perturbation in response to *Rhizoctonia solani* at 48h post-inoculation (A) and subnetwork generated by connecting possible paths between the displayed signatory metabolites (B) using the software Cytoscape and the cellular overview tool of SoyCyc (C). Representative metabolites are indicated in red color (A) and (B), whereas in the panel (C), using the Data Set S2, metabolites with KEGG identifiers are highlighted [49]

Metabolites are small molecules that are chemically transformed during metabolism and, as such, they provide a functional readout of cellular state. Unlike genes and proteins, the functions of which are subject to epigenetic regulation and posttranslational modifications, respectively, metabolites serve as direct signatures of biochemical activity and are therefore easier to correlate with phenotype. In this context, metabolite profiling, or metabolomics, has become a powerful approach that has been widely adopted for clinical diagnostics. The metabolome — typically defined as the collection of small molecules produced by cells — offers a window for interrogating how mechanistic biochemistry relates to cellular phenotype.

With developments in mass spectrometry, it is now possible to rapidly measure thousands of metabolites simultaneously from only minimal amounts of sample. In particular, recent innovations in instrumentation, bioinformatic tools and software enable the comprehensive analysis of cellular metabolites without bias. In many instances, these metabolites can be spatially localized within biological specimens with imaging mass spectrometry. The application of these technologies has revealed system-wide alterations of unexpected metabolic pathways related to phenotypic perturbations. Moreover, many of the molecules detected are currently not included in databases and metabolite repositories, indicating the extent to which our picture of cellular metabolism is incomplete. Nonetheless, the field of metabolomics has made remarkable progress within the past decade and has implemented new tools that have offered mechanistic insights by allowing for the correlation of biochemical changes with phenotype

[49].

Here we present a metabolic profiling strategy employing direct infusion Orbitrap mass spectrometry (MS) and gas chromatography-mass spectrometry (GC/MS) for the monitoring of soybean's (*Glycine max* L.) global metabolism regulation in response to *Rhizoctonia solani* infection in a time-course. Key elements in the approach are the construction of a comprehensive metabolite library for soybean, which accelerates the steps of metabolite identification and biological interpretation of results, and bioinformatics tools for the visualization and analysis of its metabolome. The study of metabolic networks revealed that infection results in the mobilization of carbohydrates, disturbance of the amino acid pool, and activation of isoflavonoid, α -linolenate, and phenylpropanoid biosynthetic pathways of the plant. Components of these pathways include phytoalexins, coumarins, flavonoids, signaling molecules, and hormones, many of which exhibit antioxidant properties and bioactivity helping the plant to counterattack the pathogen's invasion. Unraveling the biochemical mechanism operating during soybean-*Rhizoctonia* interaction, in addition to its significance towards the understanding of the plant's metabolism regulation under biotic stress, provides valuable insights with potential for applications in biotechnology, crop breeding, and agrochemical and food industries.

Metabolomics is a robust bioanalytical tool for the comprehensive analysis and monitoring of plant metabolome [50-53]. However, its application for monitoring the regulation of the global plant metabolism in response to biotic stresses is still in its infancy, receiving increasing attention. This, could provide valuable information for applications in plant biotechnology, biomarker-assisted selection, and agrochemical, food, and pharmaceutical industries [54], and in turn could boost agricultural production. The recent advances in bioanalytical protocols, analyzers, metabolite databases, and bioinformatics software enable the recording of a vast number of chemical features in the analyzed plant samples, whose identification and biological interpretation is challenging. Moreover, there is an increasing demand for standardization of data reporting for large-scale metabolomics, which will help researchers to cross-reference results from different studies with profound benefits. Within this context, we have undertaken the task of developing a highthroughput metabolomics / bioinformatics protocol for the robust dissection of plant-fungal pathogen interaction using the pathosystem; soybean [*Glycine max* (L.) Merrill, Leguminosae] and its soil-borne fungal pathogen-*Rhizoctonia solani* Ku^{hn} (anastomosis group 4, AG4). For the analysis of soybean's metabolome direct infusion Orbitrap mass spectrometry (DIMS) and gas chromatography-MS (GC/MS) analyzers were employed, which exhibit complimentary capabilities for metabolite detection and identification.

Regulation of the Phenylpropanoid Biosynthetic Pathway. The general disturbance of soybean metabolism is evident by the fluctuation of metabolites of the phenylpropanoid pathway, which plays an important role in plant's physiology, including defense responses, and several of its steps are CyP450-dependent. Actual promising approach to improve soybean innate resistance to biotic stresses is increasing of lignin biosynthesis as the natural anti-microbial compounds by genetic engineering of the **phenylpropanoid pathway**. The general phenylpropanoid metabolism generates an enormous array of secondary metabolites based on the few intermediates of the shikimate pathway as the core unit. In recent years, various excellent reviews summarized the current knowledge on structural genes involved in phenylpropanoid, specifically lignin and flavonoid formation, regulatory transcription factors, hormonal control of the whole pathways by jasmonate or auxin and evolution of pathway genes from primary metabolism [55, 56].

Transgenic approaches. Transgenic regulation of major lignin genes could led to increase lignin biosynthesis, content, increased dry matter, and improved natural innate barrier to micropathogene penetration. Increasing of lignin in soybean crop by genetic engineering is likely one of the most effective ways of combar to biotic stress and diseases. Transgenic approaches allowed to go from the study of stress tolerance mechanisms in plants to crop genetic improvement [46, 57-62]. Use of modern molecular biology tools for elucidating the control mechanisms of stress tolerance, and for engineering stress tolerant plants is based on the expression of specific stress-related genes. To date, successes in genetic improvement of environmental stress resistance have involved manipulation of a single or a few genes involved in signaling/regulatory pathways or that **encode enzymes involved in these pathways** [4, 5, 63]. There is, therefore, a need to find a compatible plant transformation methodology.

MYB transcription factors. Over the last few years, the regulation of some genes of the lignin

biosynthetic pathway has begun to be elucidated by the isolation and characterization of R2R3-MYB factors, whose belonging to different subgroups has been described as regulators of lignifications. For example, the *PAP1* gene from *Arabidopsis* encodes an R2R3-MYB which, when over_expressed in *Arabidopsis*, alters lignin biosynthesis. The *Pinus taeda* R2R3-MYB TFs *PtMYB1* and *PtMYB4* that can bind to DNA motifs known as AC elements, which are ubiquitous in the promoters encoding lignin biosynthetic enzymes, can alter the accumulation of transcripts corresponding to genes encoding lignin biosynthetic enzymes in transgenic plants. The advantages of using transcription factors for metabolic engineering in plants. Transcriptional activators and repressors, including the chimeric repressors generated by CRES-T, are useful tools for the genetic engineering of metabolic pathways (Figure 5). There are about 10 specific enzymes and 3-5 non-specific enzymes involved in lignin biosynthesis in different plant species. And the eucalyptus *EgMYB2* is also able to regulate transcription of two lignin biosynthetic genes, *CCR* and *CAD*, in both transient and stable expression assays. Recently two new maize R2R3-MYB transcription factors, *ZmMYB31* and *ZmMYB42*, have been reported to down-regulate both the *Arabidopsis* and the maize *COMT* genes. Furthermore, over-expression of the two genes also affects the expression of other genes of the lignin pathway and produces a decrease in lignin content of transgenic plants. These examples illustrate the potential for the involvement of R2R3-MYB proteins in the regulation of lignification in xylem [64-67].

Phenylpropanoid biosynthetic pathway. Phenylpropanoid metabolism is one of the three main types of secondary metabolism involving modification of compounds derived initially from phenylalanine, which is now well understood. As the first step, phenylalanine is deaminated to yield cinnamic acid by the action of phenylalanine ammonia lyase (PAL). Cinnamic acid is hydroxylated by cinnamate-4-hydroxylase (C4H) to 4-coumaric acid, which is then activated to 4-coumaroyl-coenzyme A (CoA) by the action of 4-coumarate-CoA ligase (4CL). Then it is divided into two major pathways – the flavonoid biosynthesis pathway and the lignin biosynthetic pathway. To date, most R2R3-MYB proteins have been reported to play a major role in the regulation of secondary metabolism, such as the phenylpropanoid biosynthetic pathway.

Lignin is the most prominent polymer on Earth, besides cellulose. The various aspects of lignin and lignan formation have been summarized in a recent review by Harakava R. (2005); Davin et al. (2008); Iwase A. et al. (2009); Hall R.D. and Hardy N.W. (2012) [7, 60, 68]. These reviews also illustrates progress and experimental limitations in structural elucidation of the various forms of lignin in monocots and dicots. Due to its economical value for timber and biofuel formation, lignin biosynthesis and manipulation has been a central research focus. Lignin, after cellulose, is the second most abundant terrestrial organic polymer, accounting for up to 30% of all vascular plant tissue. Deposition of lignins reinforces plant cell walls, providing rigidity, impermeability to water, and protection against pathogens. Lignins are complex racemic aromatic heteropolymers that, in Gymnosperms, derive mainly from coniferyl alcohol and a small proportion of *p*-coumaryl alcohol, and in Angiosperms, from approximately equal parts of coniferyl and sinapyl alcohols. These monolignols are products of the phenylpropanoid metabolism, which is regulated by *MYB* transcription factor family and initiated by deamination of phenylalanine by the enzyme phenylalanine ammonia-lyase (*PAL*) (figure 3). A series of hydroxylation and *O*-methylation reactions, and conversion of side-chain carboxyl to an alcohol results in the building blocks of lignins. In the traditional view, this series of reactions occurred at the level of free hydroxycinnamic acids, but recent discoveries led to a reformulation of the pathway where hydroxycinnamic acid esters play a central role [69-70].

CHARACTERIZATION OF PLANT MYB TRANSCRIPTION FACTOR FAMILY

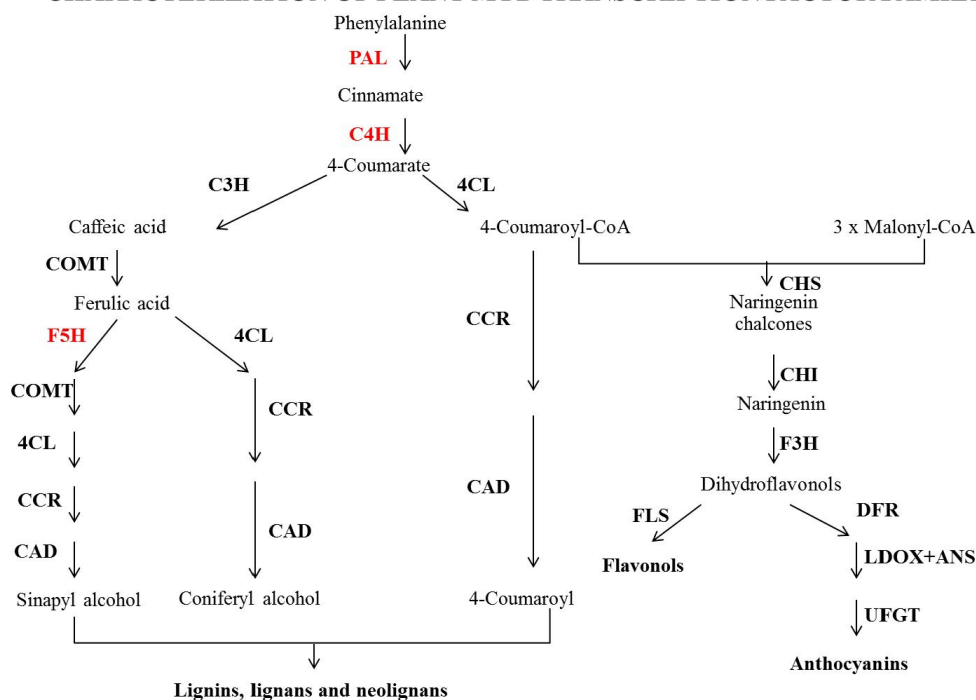


Figure 5. Diagram of enzymatic steps in pathways committed to phenylpropanoid metabolism. Abbreviations: Pal, phenylalanine ammonia lyase; C4H, cinnamate-4-hydroxylase; C3H, coumaroyl-quinic/ shikimate 3 hydroxylase; COMT, caffeic acid:5-hydroxyferulic acid O-methyl transferase; F5H, ferulate 5-hydroxylase; 4CL, 4-coumarate CoA ligase; CCR, cinamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; CHS, chalcone synthase; CHI – chalcone isomerase; F3H, flavonon 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; LDOX, leucoanthocyanidin dioxygenase; ANS, anthocyanidin synthase; UFGT, UDP-glucose: flavonoid 3-O-glucosyltransferase.

Lignin is the generic term for a large group of aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropanoids. These polymers are deposited predominantly in the walls of secondarily thickened cells, making them rigid and impervious. In addition to developmentally programmed deposition of lignin, its biosynthesis can also be induced upon various biotic and abiotic stress conditions, such as wounding, pathogen infection, metabolic stress, and perturbations in cell wall structure. Because lignin protects cell wall polysaccharides from microbial degradation, thus imparting decay resistance, it is also one of the most important limiting factors in the conversion of plant biomass to pulp or biofuels. The removal of lignin from plant biomass is a costly process; hence, research efforts are now aimed at designing plants that either deposit less lignin or produce lignins that are more amenable to chemical degradation [71].

The main building blocks of lignin are the hydroxycinnamyl alcohols (or monolignols) coniferyl alcohol and sinapyl alcohol, with typically minor amounts of *p*-coumaryl alcohol. The monolignols are synthesized from Phe through the general phenylpropanoid and monolignol-specific pathways. Phe is derived from the shikimate biosynthetic pathway in the plastid. Certain enzymes of the lignin biosynthetic pathway, namely the cytochrome P450 enzymes CINNAMATE 4-HYDROXYLASE (C4H), *p*-COUMARATE 3-HYDROXYLASE (C3H), and FERULATE 5-HYDROXYLASE (F5H), are membrane proteins thought to be active at the cytosolic side of the endoplasmic reticulum. Although metabolic channeling has been shown between PHENYLALANINE AMMONIA-LYASE (PAL) and C4H, it remains unknown whether the other pathway enzymes are also part of metabolic complexes at the endoplasmic reticulum [72-75].

Metabolomics of Plant Defense against Pathogens.

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most devastating pathogen of

soybean (figure 6).

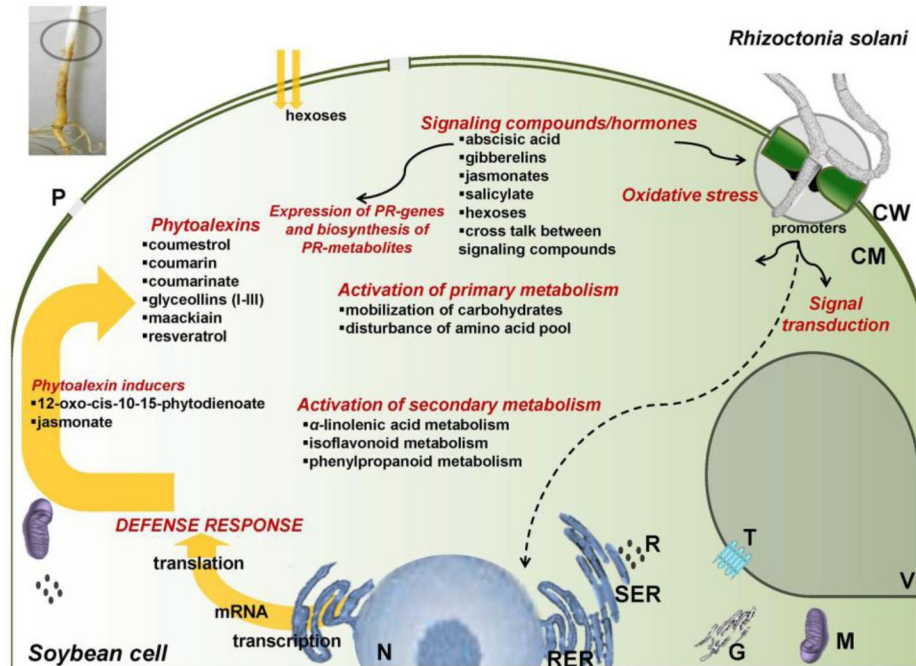


Figure 6. Proposed graphical model for the role of *Rhizoctonia solani* in activation of soybean defense mechanisms [49]

Many gene expression profiling studies have been conducted to investigate the responses of soybean to the infection by this pathogen using primarily the first-generation soybean genome array that covered approximately 37,500 soybean transcripts. However, no study has been reported yet using the second-generation Affymetrix soybean whole-genome transcript array (Soybean WT array) that represents approximately 66,000 predicted soybean transcripts. In the present works, the gene expression profiles of two soybean plant introductions (PIs) PI 437654 and PI 567516C (both resistant to multiple SCN HG Types) and cultivar Magellan (susceptible to SCN) were compared in the presence or absence of the SCN inoculum at 3 and 8 days post-inoculation using the Soybean WT array. Data analysis revealed that the two resistant soybean lines showed distinctive gene expression profiles from each other and from Magellan not only in response to the SCN inoculation, but also in the absence of SCN. Overall, 1,413 genes and many pathways were revealed to be differentially regulated. Among them, 297 genes were constitutively regulated in the two resistant lines (compared with Magellan) and 1,146 genes were responsive to the SCN inoculation in the three lines, with 30 genes regulated both constitutively and by SCN. In addition to the findings similar to those in the published work, many genes involved in ethylene, protein degradation, and phenylpropanoid pathways were also revealed differentially regulated in the present study. GC-rich elements (e.g., GCATGC) were found over-represented in the promoter regions of certain groups of genes.

Different soybean lines showed different gene expression profiles in the presence and absence of the SCN inoculum. Both inducible and constitutive gene expression profiles may contribute to resistance to multiple SCN HG Types in the resistant soybean PI lines. Ethylene, protein degradation, and phenylpropanoid pathways, as well as many other pathways reported previously, may play important roles in mediating the soybean-SCN interactions. The revealed genes, pathways, and promoter elements can be further explored to regulate or engineer soybean for resistance to SCN.

Conclusions. The main results of our research: Genetic constructions of valuable genes: transcription factor *Cs/MYB4sens.*, the main genes of lignification process - *35S/PAL*, *C₄H/F₃H*, antioxidant stress anti-ROX gene *FeSOD* have been optimized and used for soybean genetic transformation. Transgenic soybean plants of the first T₁ and second T₂ generations with integrated into genome genes of lignification

were confirmed by PCR and RT-PCR methods with transformation efficiency 5.63% in first T₁ and 75% in second T₂ generations consequently. Metabolic profiling analysis show increasing of lignin biosynthesis in transgenic soybean plants, so transition have been done from Genome to Metabolome in Post-Genomic era. Different soybean lines showed different gene expression profiles in the presence and absence of Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) inoculum. Both inducible and constitutive gene expression may contribute to resistance to multiple SCN HG Types in the resistant soybean PI lines. Ethylene, protein degradation, and phenylpropanoid pathways, as well as many other pathways reported previously, may play important roles in mediating the soybean-SCN interactions. The revealed genes, pathways, and promoter elements can be further explored to regulate or engineer soybean for resistance to SCN.

Although the biochemical basis of pathogenesis is extensively studied in plant-pathogen pathosystems, the recent developments in metabolomics now facilitate the comprehensive monitoring of the plant's metabolome and metabolism regulation in response to stimuli, and their study as a whole rather as fragmented pathways.

Additionally, although the general knowledge exists for a given plant-pathogen pathosystem, factors related to their genotypic composition could alter the final outcome. Based on its potential, the developed approach could provide new insights and could fill gaps in the knowledge related to the metabolic responses of plants during pathogen invasion.

Additionally, metabolomics data reporting and biological interpretation are facing challenges arising from the inconsistency of chemical names and lack of standardized nomenclature for metabolic pathways across public repositories. The metabolomics strategy reported here enables the robust reporting and biological interpretation of data from untargeted metabolomics experiment by providing standardized overview of the soybean's metabolism regulation during fungal infection, and could be adapted in similar studies. In addition to its significance for plant pathology, results provide information that could be exploited in genetic engineering, biotechnology, crop breeding, agriculture, food industry.

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Перспективы развития ...омикс исследований в казахстане как новый этап биотехнологии растений в пост-геномную эру

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Ключевые слова: ...омикс исследования, улучшение генома, генетическая трансформация, метаболомикс, метаболический профайлинг, лигнин, болезни, соя.

Аннотация. Следующее поколение биотехнологических культур обещает включить в себя широкий спектр продуктов, которые обеспечат преимущества как для фермеров, так и потребителей, с целью удовлетворения глобальных сельскохозяйственных проблем. Эти продукты, скорее всего, будут связаны с регулированием ключевых эндогенных путей в растениях и приведут к повышению количественных признаков растений, таких как качество, фотосинтез, устойчивость к биотическим и абиотическим стрессам.

Появление новых «omics» технологий - таких как геномика, протеомика и метаболомика, теперь позволяет исследователям идентифицировать генетику реакций растений на стресс. На сегодняшний день, успехи в генетическом улучшении устойчивости растений к стрессам среды обитания включают манипуляции одним или несколькими генами, вовлеченными в сигнальные / регуляторные пути, или кодирующими ферменты, участвующие в ключевых метаболических путях. В последние годы, в различных превосходящих обзорах обобщены современные знания о структуре генов, участвующих в фенилпропаноидном цикле, в частности, в биосинтезе лигнина и флавоноидов, регуляции транскрипционных факторов, гормональном контроле основных метаболических путей жасмонатами или ауксинами и эволюции генов ключевых метаболических путей от первичного метаболизма. Генная

инженерия ключевых метаболических путей является мощным инструментом улучшения сельскохозяйственных культур путем биотехнологии нового поколения в постгеномную эру.

Болезни сои во всем мире являются одной из серьезных проблем, снижающих урожай сельскохозяйственных культур до 11% - 30% от общего объема производства. Во многих странах контроль болезней сои ограничивается только сельскохозяйственными технологиями. Основной идеей нашего исследования является улучшение природной естественной устойчивости сои к биотическим стрессам с помощью генной инженерии фенилпропаноидного цикла, а именно - введение в сою ключевых генов, вовлеченных в биосинтез лигнина, - соединения, участвующего в широком круге физиологических процессов, таких как рост растений, водный обмен, а также обеспечивающего непроницаемость клеточных стенок - натуральный механический барьер для защиты растений от проникновения патогенов.

Предлагаемый подход борьбы с болезнями сои включает метод молекулярного клонирования и конструирования транскрипционного фактора *PTMub*, ключевых генов, участвующих в биосинтезе лигнина: *PAL*, *C4H / F5H*, *CAD*, *COMT* и т.д., с последующей идентификацией генов и их секвенированием в сотрудничестве с UIUC, США; оптимизацию разработанной нами ранее технологии *germ-line* генетической трансформации; скрининг и молекулярную детекцию трансгенов с помощью ПЦР и RT-ПЦР; анализ физиологических и биохимических последствий интродукции этих ценных генов в сою; анализ параметров биосинтеза лигнина и метаболического профайлинга трансгенных растений; анализ устойчивости трансгенов к микробиотическим стрессам; методы характеристики фенологии, морфологии и продуктивности.

Полученные результаты: 1. Генные конструкции основных генов, участвующих в биосинтезе лигнина, подготовленные к интродукции в сою. 2. Оптимизированная *germ-line* технология генетической трансформации сои. 3. Молекулярно подтвержденные трансгены сои T₁ - T₂ поколения с генами интереса. 4. Биохимическое подтверждение повышения биосинтеза лигнина, метаболический профайлинг. Таким образом, осуществлен переход от Генома к Феному в пост - геномной эре.

ПСТ-ГЕНОМДЫҚ ДӘУІРІНДЕГІ ...ОМІКС ЗЕРТТЕУЛЕРІНІҢ ӨСІМДІКТЕР БИОТЕХНОЛОГИЯСЫНДА ЖАҢА КЕЗЕҢІ РЕТІНДЕ ҚАЗАҚСТАНДА ДАМУ ПЕРСПЕКТИВАЛАРЫ

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Кілт Сөздер омикс зерттеулері, геномдық дамуы, генетикалық трансформация, метаболомикс, метабололикалық профайлинг, лигнин, аурулар, майбұршақ.

Аңдатпа. Ауыл шаруашылығы өндірісінің алдында тұрған ғаламдық мәселелерді шешу мақсатында, фермерлер мен тұтынушылардың артықшылықтарын қамсыздандыру үшін, құрамына көптеген өнімдер енетін кең ауқымды биотехнологиялық дақылдардың келесі ұрпағы көптеп саналады деген тұжырым бар. Бұл өнімдерді алуды, өсімдіктің негізгі эндогенді жолдарының реттелуімен байланыстырып, өсімдіктің сапалық және фотосинтез көрсеткіші мен биотикалық және абиотикалық торығуларға төзімділігі сияқты белгілердің сандық көрсеткіштерінің артуынан болады деп күтілуде.

Геномика, протеомика және метаболомика сияқты - жаңа «omics» технологиялардың пайда болуы зерттеушілерге өсімдіктердің торығуға беретін жауабының генетикасын танып білуге мүмкіндік береді. Бүгінгі күнге, өсімдіктердің торығуға төзімділігін генетикалық жақсарту жолындағы жетістіктер, сигналды/реттеуші жолдарға қатысты гендерді немесе негізгі метаболитті жолдарға қатысатын ферменттерді кодтайтын бір немесе бірнеше гендердің айналымы эрекеттері арқылы болып отыр. Соңғы жылдары, әр түрлі жоғарғы санатты шөлуларда фенилпропаноидты цикл, оның ішінде, лигнин мен флавоноидтар биосинтезінде, транскрипті факторлардың реттелуінде, жасмонаттармен немесе ауксиндармен гормоналды тексерілетін негізгі метаболитті жолдардың және алғашқы метаболиттерден басталатын метаболитті жолдардың гендерінің эволюциясына қатысатын гендердің құрылымы туралы заманауи білімдер жинақталған. Негізгі метаболитті жолдардың гендік инженериясы, постгеномды замандағы биотехнологияның жаңа ұрпағы арқылы ауылшаруашылық дақылдарды жақсарту үшін пайдаланылатын қуатты құрал болып табылады.

Май бұршағының аурулары, дүние жүзінде ауылшаруашылығы дақылдарының өнімділігін 11% - 30% дейін төмендететін негізгі мәселелердің бірі. Көптеген елдерде май бұршағы ауруларымен күрес тек ауылшаруашылығы технологияларын пайдаланумен шектеледі. Біздің зерттеулеріміздің негізгі түйіні, май бұршағының биотикалық торығуға табиғи төзімділігін - көптеген физиологиялық процесстерге, оның ішінде өсімдіктің өсуіне қатысатын, жасуша қабырғаларының өткізбеушілігін табиғи механикалық қорғаушы кедергі құрай отырып арттыратын, осылайша зиянды потогендердің енуін тежейтін - лигнин биосинтезіне қатысатын гендерді енгізу арқылы, фенилпропаноидты циклдің генетикалық инженериясын пайдалана отырып арттыру.

Ұсынылып отырған май бұршақ ауруларына қарсы күрес: молекулярлы клондау әдісін және *PTMub* транскрипционды факторын, лигнин биосинтезіне қатысатын негізгі гендерді: *PAL*, *C4H / F5H*, *CAD*, *COMT* және т.б. конструциялау, сонымен оларды UIUC, АҚШ қосылып бірдейлестіру және секвенирлеу; бұрынғырақ өзіміз құрастырған *germ-line* генетикалық трансформациялау технологиясын оңтайластыру; ПЦР және RT-ПЦР көмегімен трансгендерді скринингілеу және молекулярлы детекциясын жасау; май бұршағына енген гендердің физиологиялық және биохимиялық өзгеруін; лигнин биосинтезі параметрлерін және трансгенді өсімдіктердің метаболиттік профайлингін, трансгендердің микробиотическілерге төзімділігін талдау; фенологиялық, морфологиялық, өнімділігін сипаттау әдістерін пайдаланудан тұрады.

Алынған нәтижелер: 1. Май бұршағына енгізуге дайындалған, лигнин биосинтезіне қатысатын негізгі гендердің конструциясы. 2. Оңтайластырылған май бұршағының *germ-line* генетикалық трансформациялау технологиясы. 3. Май бұршағының бойында қызықтатын гендері бар T₁ - T₂ трансгенді ұрпақтары. 4. Лигнин биосинтезінің, метаболиттік профайлингтің артуының биохимиялық расталуы. Осылайша, пост - геномды заманда Геномнан Феномға өту жүзеге асты.

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