

APPLICATION OF METABOLOMIC ANALYSIS IN EXPLORATION OF PLANT GENETIC RESOURCES

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The article addresses the issues of using metabolomic analysis to study genetic resources of cereal crops in order to (1) determine phylogenetic linkages between species (the degree of domestication); (2) within species to describe genetic diversity according to its responses to biotic and abiotic stressors and biochemical characteristics (chemical compounds) determining food, feed and technological quality indicators; and (3) select the most resistant and highest-quality genotypes for complex breeding use.

Key words: *cereal crops, Fusarium head blight, mycotoxins, gas chromatography, mass spectrometry, stress resistance, nutritional value.*

ROLE OF PLANT GENETIC RESOURCES IN PROVIDING THE POPULATION WITH HIGH-QUALITY HEALTHY FOOD

In a series of our recent publications dedicated to the role of plant genetic resources in providing the population with high-quality and healthy food (Shelenga *et al.*, 2014; Konarev *et al.*, 2015; Shelenga *et al.*, 2018), we paid homage to N. I. Vavilov for his prophetic insight. In 1922, he organised research aimed at biochemical and technological quality testing of the N. I. Vavilov All-Russian Institute of Plant Genetics Resources (VIR) global collection in the biochemistry laboratories, with an accent on bioactive compounds (BAC) and technological assessment in order to supply the population with not just food products, but high-quality ones or, as termed in 2018, with a healthy diet. VIR's biochemists paid special attention to secondary metabolites or bioactive compounds (Konarev, 1994). It should be mentioned here that 80 years later, plant breeding for quality (bioactive substances) food has been declared a priority trend by the worldwide scientific community. Thus, we do not have any reason to doubt the relevance of further development of the ideas generated by VIR's founder.

The breeding value of any source material is determined by characteristics ensuring resistance to biotic and abiotic environmental factors, food or feed qualities, and technological properties of future cultivars. Until recently, a top breeding priority for the development of crop cultivars was mainly yield. Quality requirements were, as a rule, reduced to more general biochemical parameters, such as protein, lysine and starch content, and a number of technological characters. At the same time, the concept of a healthy diet was promoted, the success of which depended on the level of fundamental knowledge about metabolites and metabolic processes in humans, on the one hand, and substances received from plant-based food, on the other.

Changes occurring in breeders' work with source and breeding materials are of interest not only to the breeders themselves, but also to the experts in allied disciplines — geneticists, morphologists, biochemists, physiologists, nutritionists, etc. (Perkowski *et al.*, 2012; Gu *et al.*, 2015; Sánchez-Martín *et al.*, 2015). Both practical and theoretical aspects of the problem need to be considered in all cases.

METABOLOMIC APPROACH IN INVESTIGATION OF PLANT GENETIC RESOURCES

Essentially new possibilities opened up with the development of metabolomics. According to the present-day conceptions, the metabolomic profile is an overall result of the cell's activity on molecular-genetic and biochemical levels. Analysis of metabolomic profiles makes it possible to identify biochemical markers of biological processes. All this facilitates complex evaluation of the processes in the organism of a plant, animal or microbe in compliance with the principles of systemic biology (Afonnikov and Mironova, 2014).

In recent years, metabolomic profiles have become a called-for diagnosing tool in biology and agricultural science, used to phenotype species or varieties, analyse resistance and quality characters, facilitate breeding practice, etc. (Schauer and Fernie, 2006; Langridge and Fleury, 2011; Balmer *et al.*, 2013; Smolikova *et al.*, 2015).

During the last decades, the methodological basis underpinning present-day research with the above-mentioned compounds has been gas-liquid and liquid (high-pressure) chromatography, especially effective in combination with mass spectrometry (Lokhov and Archakov, 2008; Shulaev *et al.*, 2008). Thus, modern molecular biological research is characterised by application of various techniques enabling the researcher to study plant organisms on genomic and proteomic levels as well as on the level of the metabolome (Harrigan *et al.*, 2005; Hollywood *et al.*, 2006; Shulaev, 2006).

The most important biochemical traits in cultivated plants, characterising their valuable alimentary, medical and biological properties, are secondary metabolites of plants and the main substances of primary metabolism — polysaccharides, proteins and lipids. In this aspect, the task of plant genetic resources conservation and their sustainable utilisation in order to raise the quality of human life, which is primarily based on the quality of food, has become more and more vital.

Plant secondary metabolites are carriers of pharmacological and toxic properties, and predecessors of mutagenic and teratogenic compounds — substances rendering a considerable effect on the development of gut microbiota. Eleven groups of non-alimentary substances with an experimentally proven pharmacological effect have been identified (dietary fibres, oligosaccharides, polyfunctional alcohols, phenolic compounds, terpenes and terpenoids, phospholipids, glycosides, vitamins, etc.) (Krasilnikov, 2015).

One of the tasks of metabolomics is to study the organism's response to the environment. Any exposure of an organism leads to multiple changes in concentrations of various metabolites to maintain its homeostasis. Normally, intracellular metabolites retain dynamic equilibrium with the environment's metabolites. Analysis of the obtained metabolic profiles makes it possible to capture a distinctive "fingerprint" reflecting the organism's physiological condition (Sitkin *et al.*, 2013).

Cereal crops frequently serve as material for metabolomic research, as they have been persistently searched for optimum sources of a balanced diet. Studying metabolites in cereal crops facilitates evaluation of the components with low concentration coupled with high biological activity, such as phenolic compounds (PCs), polyols, free amino acids, phytosterols and a number of organic acids (Žilić *et al.*, 2011; Björck *et al.*, 2012).

Study of metabolites in living organisms will help to reveal the effect of genetic modifications or biotic and abiotic stressors upon them (Khakimov *et al.*, 2014a).

Thus, metabolomics is a promising approach to the search for linkages between biochemical components and genetic features in cereal crops, and can open new possibilities for quality-targeted breeding programmes (Fernie and Schauer, 2009).

For example, Japanese scientists recently chose gramin and benzoxazinons, the secondary metabolites responsible for disease and pest resistance in many cereal crops, as the object for such kind of screening (Kokubo *et al.*, 2017).

The metabolomic approach to studying fungus diseases must meet several criteria: precision in identification of components; statistical reliability of the quantitative assessment of the studied variability, i.e. significance of the changes in the content and composition of metabolites in resistant and susceptible "plant-pathogen associations" (Choo, 2016). *Fusarium* resistance is controlled by the genes of more than a hundred loci of quantitative characters (Lemmens *et al.*, 2005; Buerstmayr *et al.*, 2009).

In the process of their evolution, plants have developed diverse responses to the effect of pathogens, including biochemical ones, and undergone structural transformations in their organs and tissues. Host plants are able to produce chemical compounds of varied nature, which inhibit the development of a pathogen. Chemical factors, with the help of which a plant protects itself from diseases, are diverse in composition, conditions of their formation, and mechanisms of toxicity. Plants possess not only chemical diversity of protective compounds, but also various strategies of their deployment, thus making shielding barriers in all stages of the parasite's advancement (Dyakov, 2012).

An analytical approach based on chromatography combined with mass spectrometry opens up more opportunities for a researcher to understand biochemical processes in the plant-pathogen interactions. It provides information on the changes in many chemical compounds (metabolites), including those whose synthesis is induced by the pathogen (Fiehn *et al.*, 2000; Hill, Roessner, 2013; Loskutov *et al.*, 2017).

ROLE OF INDIVIDUAL METABOLITES IN BUILDING UP RESISTANCE TO STRESS FACTORS (*FUSARIUM*)

Analysing the consequences of a plant's exposure to a pathogen is one of the approaches to solve the problem of

resistance. Among the ways used by a plant to protect itself, a significant role is performed by chemical factors. It is important that biochemical processes can be characterised using measurable parameters (amount of toxins produced by the parasite, content and quantity of metabolites produced by the plant in response to the stress, etc.).

Fusarium head blight is a fungal disease attacking cereal crops, including wheat, barley and oat (Gagkaeva *et al.*, 2011; 2014), which results in harvest losses and is regarded as a global threat to food security (Chakraborty and Newton, 2011). The most widespread species causing this disease is *Fusarium graminearum* Schwabe, which produces deoxynivalenol (DON). This mycotoxin is considered the main factor of the fungus' aggressiveness, inhibiting *inter alia* protein translation in eukaryotes (Pestka, 2010). *Fusarium* Link species are able to produce toxins with different aggressiveness. Among them, there are *F. culmorum* (W.G. Sm.) Sacc., a highly aggressive DON-producing pathogen, and *F. sporotrichioides* Sherb., a relatively weak pathogen producing T-2 mycotoxin (Gagkaeva *et al.*, 2014). In addition to economic loss due to yield decreases, mycotoxins of these fungi threaten the health of consumers.

The DON-induced metabolic response in grain infected with *Fusarium* includes the loss of chlorophyll (Bushnell *et al.*, 2009). Photosynthesis subsides at the infected site (Bolton, 2009). As a result, primary (carbohydrate) and secondary (nitrogen) metabolism is modified. The blockage of protein biosynthesis by mycotoxins leads to accumulation of free amino acids (Warth *et al.*, 2015).

A biochemical approach (and specifically, metabolomic one) when combined with genetic methods can provide valuable information on the plant's protective mechanisms against penetration of *Fusarium* fungi (Kluger *et al.*, 2015).

In an optimum situation, the plant may contain components "harmful" for the pathogenic fungus, such as glycosides — phenolic, cyanogenic and other compounds — and synthesise, for example, phytoalexins, as a response to microbial infection (Dyakov, 2012).

The accomplished complex study of biochemical characteristics in oat cultivars resistant and non-resistant to *Fusarium* has shown their differences, which should be taken into account while developing new resistant cultivars with high quality of grain (Konarev *et al.*, 2015).

Metabolomic analysis of oat cultivars resistant and non-resistant to *Fusarium* head blight has demonstrated a connection between the main groups of metabolites in the oat kernel and *Fusarium* head blight infection indicators (amount of DNA and concentration of toxins). An increase in the amount of DNA of *F. sporotrichioides* is correlated with an increase in the content of fatty acids in kernels and oligosaccharides and a decrease in the content of organic acids and monosugars. An increase of DNA content in *F. culmorum* was observed to be linked with a decrease in the content of phytosterols and monosugars. An increase in the

content of DON toxins in kernels was observed to be correlated with an increase of the total amount of organic and fatty acids and a decrease in amount of amino acids and polyatomic alcohols. An increase in the amounts of DON and T-2 toxins led to a reduction of the total of monosugars in the metabolite profile of oat kernels. The obtained data, which for the first time disclosed linkages of a wide range of compounds with various indices of *Fusarium* resistance in oat cultivars, are important not only to understand the nature of the resistance trait, but even more crucially, to develop methods of its diagnosis. Considering the polygenic nature of the control over trait resistance to *Fusarium* head blight and the dependence of its expression on the environment, it is necessary to study a crop's broad intra- and inter-species diversity under different growing conditions (Loshkutov *et al.*, 2019a).

In the analysed oat accessions, oxycinnamic, oxybenzoic, pipercolic and 5-hydroxypipercolic acids have been identified. Since the latter are known for their anti-*Fusarium* activity (Cuperlovic-Culf *et al.*, 2016), it becomes possible to use collections for selecting accessions with the highest concentrations of these compounds as sources of potential resistance to *Fusarium*.

It should be mentioned that even earlier VIR made efforts to clarify the role of specific substances in building up resistance to biotic and abiotic environmental factors. That was the aim of the research on the role of endophytic fungi's alkaloids in the resistance of forage grasses to pest insects and in their winter hardiness (Shelenga *et al.*, 2006).

In recent years, the metabolomic approach has been used to solve the ever growing range of problems, including the evaluation of cultivar-specificity in seeds, the task of utmost importance in the Vavilov Institute (Smolikova *et al.*, 2015).

Assessment of the content of the known bioactive substances (BAS) and searching for new ones require screening of plant genetic resources. A logical consequence of these processes is breeding for the traits in question, which in its turn has required development of methodological approaches to the analysis of such compounds in the source and breeding materials, and in the end products resulting from cultivation of the released cultivars.

CHANGES IN THE OAT METABOLOMIC SPECTRUM IN THE PROCESS OF "DOMESTICATION"

The urgency and importance of BAS-targeted biochemical research was recognised at the Vavilov Institute of Plant Genetic Resources (VIR) as early as in the 1930s. Such research was conducted by VIR in the following years as well (Ermakov *et al.*, 1969; Anonymous, 1987; Konarev and Khoreva, 2000). The results of the biochemical screening of the global collection have not lost their significance up to the present time. Moreover, in recent years they served as a

platform for a number of international projects launched to address the problems of functional nutrition.

The metabolomic approach, with its coverage of a wide range of compounds, is able to provide information for a more profound understanding of the processes that introduce plant forms into cultivation or the breeding work carried out already at the level of metabolic processes as a whole.

Common oat (*Avena sativa* L.) is one of the most promising and much-in-demand crops due to a whole number of its valuable traits that meet the requirements for “functional nutrition” products and favour its use for fodder production and disease prevention purposes (Loskutov, 2007; Leonova *et al.*, 2008; Loskutov and Rines, 2011). Oat grain is distinguished for its proteins, better balanced in their amino acid composition than those in other cereals, and particularly for the content of essential amino acids (arginine, histidine, lysine and tryptophan) (Loskutov, 2007; Konarev *et al.*, 2015). Oat plays an important role as a substitute for wheat in gluten-free food production for those intolerant to wheat protein or suffering from celiac disease (Alpatyeva *et al.*, 2004).

An obstacle on the way towards producing new oat (and not only oat) cultivars, competitive on the market because of their high nutritional, fodder and technological qualities, is the methodological problem of simultaneously making such cultivars resistant to biotic and abiotic environmental stressors.

It is known that crop wild relatives, along with representatives of kin species, are inexhaustible sources of properties valuable for prospective new cultivars. VIR holds a rich collection of wild oat species (around 2000 accessions), which has been studied for many years, and this study also included biochemical research on such basic traits as quality, resistance to biotic and abiotic environmental factors, etc. (Konarev and Khoreva, 2000; Loskutov, 2007; Leonova *et al.*, 2008; Loskutov and Rines, 2011; Konarev *et al.*, 2015). However, as far as we know, neither metabolomic analysis of wild oat species nor their comparison with cultivated oat has been made, at least on a large scale.

Comparing metabolomic spectra in wild species and cultivars has led to the following principal conclusions. A number of metabolites have been identified, the content of which is changing in the process of “domestication”, thus showing differences between wild oat species and oat cultivars. Among them, factors of healthy nutrition like oleic acid, glucose and fructose, etc., monoacylglycerols (MAG-1 C16:0 and MAG-2 C18:0) were identified, which are presumably associated with adaptability to environmental stress factors, including fusarium (Žilić *et al.*, 2011; Loskutov *et al.*, 2017).

In the case of MAG-1C 16:0 and MAG-2 C18:0, we may speak about a certain (probably indirect) biochemical factor linked with the process of adaptation, since a number of ad-

aptation-specific properties may be lost in the course of breeding or during introduction into cultivation, and even more so, when highly specialised, intensive, state-of-the-art cultivars are being developed. This is because this process is accompanied by a decline of the cultivar population’s genetic polymorphism, typical of old landraces produced by folk breeding). According to the results of this study, a number of wild oat species may be recommended as a potential source of qualitative biochemical characters for interspecies hybridisation: diploid *A. clauda*, with an increased content of separate fatty acids and sitosterol in the kernel; diploid *A. wiestii*, with a higher content of amino acids; tetraploid *A. agadiriana*, having an increased content of separate fatty acids and sitosterol; hexaploid *A. occidentalis*, with a unique composition of fatty acids in oil and high content of MAG and sitosterol; hexaploid *A. sterilis*, with an increased content of sitosterol in oil; and hexaploid *A. fatua* and *A. ludoviciana*, with a reduced content of sucrose and high content of monosugars in kernels (Shelenga *et al.*, 2014; Loskutov *et al.*, 2017).

METABOLOMIC PROFILE’S SPECIFICITY OF COVERED AND NAKED OATS

Naked oats (with the centre of their origin and diversity in Mongolia and Northwest China) are utilised, not quite deservedly, only on a limited scale. In recent years, breeders have become much more interested in naked cultivars due to a wide range of their consumer-oriented advantages over conventional covered oats (Loskutov, 2007; 2009).

Identification of biochemical factors affecting the expression of economically valuable traits, followed by screening of source and breeding materials for the content of such metabolites, is a widespread experimental approach to understanding the mechanisms of a valuable trait’s formation. Malic, gluconic and lactic acids appear to predominate in oat kernels over other organic acids (Khakimov *et al.*, 2014b). The first of them prevailed in naked oat, and the latter two, in covered ones. However, the content of organic acids and phosphoric acid in the metabolomic profile of naked oat is higher than in covered ones (Loskutov *et al.*, 2017; Loskutov *et al.*, 2019a).

The studied oat accessions were found to contain a diverse composition of free amino acids: α -alanine, glycine, proline, serine, oxoproline, ornithine, asparagine, aspartic acid, glutamine, glutamic acid, including essential ones: threonine, leucine, valine, tyrosine, tryptophan and phenylalanine. Glutamine has the highest content among all free amino acids in covered oat accessions, while glycine and tyrosine prevail in naked oats. Tyrosine is of interest as an important component in the synthesis of growth factors (Schenck and Maeda, 2018). Glycine is a free amino acid reportedly affecting the resistance of plants to abiotic stressors, such as drought (Sánchez-Martín *et al.*, 2015).

Our data show that naked oat contains more organic acids and phosphoric acid in their metabolomic profile than in

covered oats. This difference is generated at the expense of malic, gluconic and lactic acids. As mentioned above, the first prevails in naked oat, while the latter two in covered oat. Organic acids influence many functions of the human organism. Malic acid, for example, serves as an additive to pharmaceuticals. Glycine content in naked oat is thrice higher than in covered oats. It is not out of place to be reminded of the specific role of glycine, a neurotransmitter of the inhibitory type of action, in man's metabolic processes. Naked accessions are notable for high amounts of pipercolic and 5-hydroxypipercolic acids; one of their functions is to protect plant tissues from being afflicted with *Fusarium* fungi (Abeysekara *et al.*, 2016).

Polyatomic alcohols represent a form of storage compounds, and are also produced in response to stress (Seki *et al.*, 2002; Bhandari and Nayyar, 2014; Blanch *et al.*, 2017). They include alcohols like ononitol and galactinol, which prevail in naked oat. *Myo*-inositol is recognised as one of the main polyatomic alcohols in oat kernels, and sitosterol as the major phytosterol. *Myo*-inositol plays an important role practically in all cell processes, being a predecessor of compounds like phosphatidylinositol, *myo*-inositol hexaphosphate (phytic acid), ascorbic acid, etc. and is a participant in the transfer of intercellular signals, construction of the cell wall, preservation of a bioactive phosphate, osmotic regulation, and protection of plant cells from oxidants (Loewus *et al.*, 2000; Lahiri *et al.*, 2003; Kumar *et al.*, 2010; Okazaki *et al.*, 2014). For man, *myo*-inositol is one of the vital components of a healthy diet, due to its hypolipidemic, anticarcinogenic, antidiabetic and antioxidant effects (Croze *et al.*, 2012). Phytosterols, and sitosterol with its isomers in particular, provide anticancer effect, and their regular consumption with human food reduces the risk of cardiovascular diseases (Woyengo *et al.*, 2009; Zhao *et al.*, 2009).

Sugars in naked oat are represented mainly by monosaccharides, and in covered oats by oligosaccharides, which is important when comparing the nutritive value of oats. A higher content of glucose and raffinose was found in covered oat. According to Vidigal *et al.* (2016) and Blanch (2017), higher contents of glucose, raffinose and free amino acids enhance the resistance of plant tissues to environmental stressors (see also Žilić *et al.*, 2011; Björck *et al.*, 2012; Kaur *et al.*, 2015). However, the difference in amino acid content between naked and covered oat kernels is about 1%.

Kernels of naked and covered oat differed in quantitative and qualitative composition of phenolic compounds (PCs). In covered oat, PCs were represented mainly by oxycinnamic acids, hydroquinone and methylarbutin, while those in naked oat, by oxycinnamic and oxybenzoic acids and methylarbutin. Phenolic compounds are known to play not the least role in protecting plants from insect pests and diseases, including *Fusarium* blight (Pieterse *et al.*, 2013; Sánchez-Martín *et al.*, 2015; Sharova, 2016; Bernardi *et al.*, 2018).

Covered oat has four time higher content of acylglycerols (AG) than in naked oats. It has been found that these compounds have a possible role in building up resistance to abiotic stressors in plants, and in oats in particular (Žilić *et al.*, 2011; Loskutov *et al.*, 2017).

Principal component analysis of the metabolomic profiles in naked and covered oat kernels confirmed their difference. Significance of the differences in the metabolomic profiles between naked and covered oat shown with Tukey's criterion. The covered oat's metabolome was characterised by gluconic, lactic and ferulic acids, glutamine, MAG-2 C18:3, dulcitol, *chiro*-inositol methylarbutin, hydroquinone, resorcin, glucose and raffinose; and in naked oat by malic, phosphoric, pipercolic, 5-hydroxypipercolic, palmitic, linoleic, oleic, *para*-coumaric and benzoic acids, glycine, tyrosine, MAG-2 C18:2, ononitol, glycerol, *myo*-inositol, galactinol, isofucosterol and sucrose (Loskutov *et al.*, 2019b).

The groups of naked and covered oats, in their turn, appeared non-uniform. Within the naked oat group, a subgroup with higher levels of organic and fatty acids, polyatomic alcohols, and MAG emerged (cultivars 'Sibirsky Golozernyi', 'Progress' and 'Gehl'). In the covered oat group, subgroups with a lower content of sugars (cultivars 'Freestyle', 'Elegant', 'Raven', 'Malin' and 'Zorro') and higher sugar content (cultivars 'Mirt', 'Boto', 'Auteuil' and GN 8214) were identified. Accessions with high content of phytosterols formed a separate group (naked cultivar 'Gehl', and covered cultivars 'Zalp', 'Sapsan', 'Argamak' and 'Hurdal'). The covered cultivar 'Boto' was distinguished from the other accessions by higher content of organic, fatty and free amino acids, polyatomic alcohols, phenolic compounds, and sugars (Loskutov *et al.*, 2019b).

It is known from the previous work (Harrigan *et al.*, 2005; Hollywood *et al.*, 2006; Shulaev, 2006; Yandea-Nelson *et al.*, 2015) that the metabolomic profile's specificity is engendered by biochemical composition, depending on the genotype and the environment. The fact that we have disclosed significant differences between the metabolomic profiles of naked and covered oat serves as an argument supporting the existence of genetic differentiation in the common oat subspecies. Our research has also made it possible to identify accessions with higher contents of compounds influencing dietary benefits of common oat and its resistance to stresses (naked cultivars: 'Sibirsky Golozernyi', 'Gehl', UFRGS-106150-3 and 'Progress'; covered cultivars: 'Freestyle', 'Elegant', 'Zalp', 'Sapsan', 'Argamak', 'Hurdal', 'Raven', 'Malin', 'Boto', 'Zorro' and 'Borrus') (Loskutov *et al.*, 2019b). All oat cultivars identified during the research will be dispatched to the leading Russian breeding centres working on improvement of this crop.

CONCLUSIONS

All the above-mentioned serves as a proof that the metabolomic approach opens new perspectives in the complex study of cultivated plant genetic resources and crop wild

relatives — from identifying the carriers of traits vitally important for breeders, to solving fundamental problems of plant breeding, phylogenesis, taxonomy, evolution, identification of crop genetic resources, etc. The efficacy of such an approach has been confirmed by identification of specific metabolites as participants in the domestication and breeding processes promoting the formation of resistance traits. All this promises palpable opportunities in managing the processes of the development of valuable properties (in ontogenesis, in generations) and the creation of diagnostic test systems for identification of their carriers (Konarev *et al.*, 2015; Loskutov *et al.*, 2017).

Thus, the following basic conclusions may be made with a high degree of probability on the basis of metabolomic analysis. It has been confirmed on a new methodological level that the studied crops are carriers of economically important breeding traits, with a broad scope of their expression (biotic and abiotic), which makes them a reliable reserve for the development of cultivars that will meet the present-day requirements of food and feed production industries, healthy diets and clinical nutrition, and highly productive and environmentally safe agriculture.

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METABOLOMISKĀS ANALĪZES PIELIETOJUMS AUGU ĢENĒTISKO RESURSU IZVĒRTĒŠANĀ

Rakstā apkopota informācija par metabolomiskās analīzes pielietojumu graudaugu ģenētisko resursu pētījumos ar mērķi noteikt filoģenētiskās saites starp sugām, ģenētisko daudzveidību sugas iekšienē attiecībā uz reakciju uz biotiskiem un abiotiskiem stresiem, kā arī lai izdalītu vērtīgākos genotipus kompleksai selekcijai uz izturību un augstu kvalitāti.