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Main subject

Molecular mechanisms of antioxidative defence of plant cells

Main topics

1. Metabolism of reactive oxygen and nitrogen species in plants 2. Stress induced autophagy in plants 3. Plant membrane sterols

Main achievements for 2009-2014

1. Screening study of the mechanisms of the abiotic stress-induced formation and metabolism of reactive oxygen (ROS) and nitrogen (RNS) species is performed in the rage of plant species, such as wheat roots and leaves, seeds of wild and crop species, and also bryophytes and symbiotic organisms lichens. It is found that fast stress response is supported by the activity of pre-existing redox enzymes of cell wall and plasma membrane. Apoplastic peroxidases, which are responsible for the stress-induced superoxide formation in the apoplast of wheat and chestnut seeds, have been identified. Proteomic studies demonstrated the multiplicity of

isoforms of apoplastic peroxidases with pro- and antioxidative properties. Kinetic analysis of peroxidases from bryophytes *Dumortiera hirsuta* and *Anthoceros natalensis*, which are sisters of higher plants, showed that the formation of ROS by peroxidases is an evolutionary ancient process, which can strengthen plant resistance and facilitate their successful colonization of various ecological niches. First experimental evidence of the lignolytic activity in lichens is obtained. This activity is provided by redox enzymes, and in particular by peroxidases. Peroxidase of lichens in Suborder Peltigerineae possesses high redox potential and is able to oxidaze recalcitrant substrates, including phenolic metabolites of competing lichen species. Altogether, these results contribute to deciphering the redox mechanisms, which control stress response and resistance of plants.

2. The features of autophagic degradation of oxidized macromolecules and damaged organelles in plant cells during stress have been characterized. The main stages of the formation of autophagosomes containing the fragments of cytoplasm and organelles were identified. Induction, progression and governing capacity of macroautophagy in plants during oxidative stress was demonstrated. Analysis of the expression profile of autophagic genes *ATG4*, *ATG6* and
ATG8
by real-time PCR showed that treating plants with prooxidants, mitochondrial poisons and wounding cause up-regulation of the gene expression in wheat roots. Conservative and variable domains of
Ta
ATG4
and
TaATG8
genes were found by bioinformatic analysis. Two copies of
Ta
ATG8
g gene were annotated in GenBank (KJ740609 and KJ740610). By using bioinformatics and computer modeling, the spatial structure of autophagic proteins ATG4 and ATG8g in wheat was characterized and specific sites, which are essential for the interaction of these proteins with ligands during the formation of autophagosomes, have been identified. Recombinant wheat ATG8g protein was successfully expressed in
E.
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oli
. New knowledge of the mechanisms of autophagic degradation of oxidative proteins and damaged organelles in plants contributes to the revealing the mechanisms of stress resistance of plants.

3. The role of membrane sterols as the structural macromolecules of plasma membrane and the components of lipid microdomains has been studied. Unlike animals and fungi, plants have the diversity of molecular species of sterols. By using chromatography and mass-spectrometry, we study the composition of membrane lipids and the changes in the ratio of sterol molecular species, in particular campesterol and sitosterol, in plants in response to oxidative, wound and cold stresses. Analysis of membrane lipids during abiotic stresses and sterol depletion demonstrated the occurrence of cross-talk between sterols and glycoceramides. It was found that sterol depletion causes distinctive changes in the lipid composition, membrane permeability, oxidative stress, and the formation of autophagosomes in cells depending on the type of endogenous sterol binding by nystatin and methyl-β-cyclodextrin. Gene structure of 24C-sterol methyltransferase, a key enzyme of plant sterol biosynthesis, was analyzed. Conservative and variable domains, including stress-responsive motifs, were found in the coding and regulatory regions of the gene. Up-regulation of *TaSMT1* expression was discovered in wheat roots in response to oxidative, wound and cold stresses. These results confirm that the enzymes of sterol biosynthesis are involved in stress response of plants.

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Collaboration

Ilse Kranner (University of Innsbruck, Austria) - redox signaling in seeds Richard Beckett (University of KwaZulu-Natal, Republic of South Africa) - redox activity in lower plants

Sabine Lüthje (University of Hamburg, Germany) - identification of apoplastic peroxidases

Christiane Liers, Martin Hofrichter (International Graduate School of Zittau, Germany) – identification of redox enzymes in lichens

Mariana Sottomayor (University of Porto, Portugal) – analysis of gene sequences of plant peroxidases

Kurt Fagerstedt and Olga Blokhina (University of Helsinki, Finland) - ROS and NO production in transformed Arabidopsis plants

Kotlova E.R. (Komarov Botanical Institute, RAS, Saint-Petersburg) - identification of membrane lipids

Khalil Gainutdinov (Zavoisky Physical-Technical Institute, Kazan) - EPR studies of reactive nitrogen species

Irina Ryzhkina (Institute of Organic and Physical Chemistry, Kazan) - aggregation of synthetic compounds and biological macromolecules

PhD theses defended in 2008-2014

Dmitrieva Svetlana “Structural and functional changes in the cells of wheat roots in conditions of oxidative stress”, 2008 Ryabovol Victoria “Morphological, biochemical and molecular characteristics of autophagy in *Triticum aestivum* roots under stress”, 2014

Pedagogical activity

Chairing the State Qualification Committee in KFU (Karimova F.G.) Chairing the State Qualification Committee in KSTU (Minibayeva F.V., Karimova F.G.)

Course of lectures on Intracellular Signaling in KFU (Karimova F.G.)

Course of lectures on Photobiology in KFU (Chasov A.V.)

Supervision of students’ research and diploma projects.

Selected papers for 2009-2014

2009

- Chasov A.V., Minibayeva F.V. (2009) Effect of exogenous phenols on superoxide production by extracellular peroxidase from wheat seedling roots. Biochemistry (Moscow),

74(7): 766-774.
- Laufer Z., Beckett R.P., Minibayeva F.V., Lüthje S., Böttger M. (2009) Diversity of laccases from lichens in Suborder Peltigerineae. Bryologist, 112(2): 418-426.
- Minibayeva F., Kolesnikov O., Chasov A., Beckett R.P., Lüthje S., Vylegzhanina N., Buck F., Böttger M. (2009) Wound-induced apoplastic peroxidase activities: their roles in the production and detoxification of reactive oxygen species. Plant, Cell & Environment, 32: 497-508.

2010

- Minibayeva F.V. (2010) Redox signals in plant cells under stress: the focus on the apoplast. *In*: Cellular signaling. Fen, Academy of Sciences of RT, Kazan, Ed. A.N.Grechkin, p. 81-89 (in Russian).
- Whitaker C., Beckett R.P., Minibayeva F.V., Kranner I. (2010) Production of reactive oxygen species in excised, desiccated and cryopreserved explants of *Trichilia dregeana* Sond. South African Journal of Botany, 76: 112-118.

- Roach T., Beckett R.P., Minibayeva F.V., Colville C., Whitaker C., Chen H., Bailly C., Kranner I. (2010) Extracellular superoxide production, viability and redox poise in response to desiccation in recalcitrant *Castanea sativa* seeds. Plant, Cell & Environment, 33: 59-75.
- Li J.L.Y., Sulaiman M., Beckett R.P., Minibayeva F.V. (2010) Cell wall peroxidases in the liverwort *Dumortiera hirsuta* are responsible for extracellular superoxide production, and can display tyrosinase activity. Physiologia Plantarum, 138: 474-484.

- Kranner I., Roach T., Beckett R.P., Whitaker C., Minibayeva F.V. (2010) Extracellular production of reactive oxygen species during seed germination and early seedling growth in *Pisum sativum*
. Journal of Plant Physiology, 167: 805-811.

- Valitova Yu.N., Kotlova E.R., Novikov A.V., Shavarda A.L., Artemenko K.A., Zubarev R.A., Minibayeva F.V. (2010) Binding of sterols affects membrane functioning and sphingolipid composition in wheat roots. Biochemistry (Moscow), 75(5): 554-561.
- Viktorova L.V., Maksyutova N.N., Trifonova T.V., Andrianov V.V. (2010) Production of hydrogen peroxide and nitric oxide following introduction of nitrate and nitrite into wheat leaf apoplast. Biochemistry (Moscow), 75(1): 95-100.
- Kranner I., Minibayeva F.V., Beckett R.P., Seal C.E. (2010) What is stress? Concepts, definitions and applications in seed science. New Phytologist, 188: 655-673.

2011

- Beckett R.P., Alyabyev A.J., Minibayeva F.V. (2011) Patterns of heat production during desiccation and rehydration in lichens differing in desiccation tolerance. Lichenologist, 43(2): 178-183.
- Valitova J.N., Minibayeva F.V., Kotlova E.R., Novikov A.V., Shavarda A.L., Murtazina L.I.,

Ryzhkina I.S. (2011) Sterol depletion by nystatin increases membrane permeability and modifies sphingolipid composition in wheat roots. Phytochemistry, 72: 1751–1759.

- Liers C., Ullrich R., Hofrichter M., Minibayeva F.V., Beckett R.P. (2011) A heme peroxidase of the ascomyceteous lichen *Leptogium saturninum* oxidizes high-redox potential substrates . Fungal Genetics and Biology, 48(12): 1139-1145.

2012

- Dmitrieva, S.A., Ponomareva, A.A., Ryabovol, V.V., Minibayeva, F.V. (2012) Effects of oxidative stress on ultrastructure and functional activity of plant mitochondria *in vivo*. Biologicheskie Membrany, 29(4): 267-275 (in Russian).

- Galeeva E.I., Trifonova T.V., Ponomareva A.A., Viktorova L.V., Minibayeva F.V. (2012) Nitrate reductase from *Triticum aestivum* leaves: regulation of activity and possible role in production of nitric oxide. Biochemistry (Moscow), 77(4): 404-410.

- Minibayeva F., Dmitrieva S., Ponomareva A., Ryabovol V. (2012) Oxidative stress-induced autophagy in plants: the role of mitochondria. Plant Physiology and Biochemistry, 59: 11-19.
- Chasov, A.V., Beckett, R.P., Minibayeva, F.V. (2012) Peroxidases of Anthoceros natalensis, an evolutionary precursor of vascular plants. Doklady Biological Sciences 447 (1): 357-359.
- Beckett R.P., Minibayeva F.V., Liers C. (2012) Occurrence of high tyrosinase activity in the non-Peltigeralean lichen *Dermatocarpon miniatum* (L.)W. Mann. Lichenologist, 44(6): 827–832.

2013

- Beckett R.P., Minibayeva F.V., Liers C. (2013) On the occurrence of peroxidase and laccase activity in lichens. Lichenologist , 45(2): 277–283.
- Газизова Н.И., Петрова Н.В., Каримова Ф.Г. (2013) Влияние вольфрамата на рост корней гороха и фосфорилирование белков по тирозину. Физиология растений, 60(6): 819– 828.

2014

- Valitova J., Sulkarnayeva A., Kotlova E., Ponomareva A., Mukhitova F.K., Murtazina L., Ryzhkina I., Beckett R., Minibayeva F. (2014) Sterol binding by methyl-β-cyclodextrin and nystatin – comparative analysis of biochemical and physiological consequences for plants. FEBS Journal, 281: 2051–2060.
- Beckett R.P., Minibayeva F.V., Vinogradova A., Liers C. (2014) Hydration can induce laccase and peroxidase activity in Peltigeralean and non-Peltigeralean lichens. Lichenologist, 46(4): 589–593.
- Sulkarnayeva A.G., Valitova J.N., Mukhitova F.K., Minibayeva F.V. (2014) Stress-induced changes in membrane sterols in wheat roots. Doklady Biochemistry and Biophysics, 455: 53-55.
- Chasov A.V., Minibayeva F.V. (2014) Methodological approaches for studying apoplastic redox activity: 1. Mechanisms of peroxidase release. Russian Journal of Plant Physiology, 61(4): 556–563.
- Chasov A.V., Minibayeva F.V. (2014) Methodological approaches for studying apoplastic redox activity: 1. Regulation of peroxidase activity. Russian Journal of Plant Physiology, 61(5): 626–633.
- Ryabovol V.V., Minibayeva F.V. (2014) Autophagic proteins ATG4 and ATG8 in wheat: structural characteristics and their role under stress conditions. Doklady Biochemistry and Biophysics, 458: 179–181.