Laboratory of Enzyme Chemistry

Laboratory head

Professor Akhunov Ali Akhunovich, Doctor of Biological Sciences

Contacts: Tel.: +998 (90) 351 12 06, e-mail: ali.akhunov@gmail.com

Laboratory of chemistry of enzymes was founded in 1987 on the basis of a group of biochemical analysis under Department of Zoo-Phytotoxins at the Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences. Study on biologically active agents of plant and animal origin is the principle direction of research. Components of the kallikrein-kinin system were found in the venoms of spiders for the first time making possible explanation of some principles of toxicological effect of the venoms on a living organism. Kininase, a homogenous enzyme with knowing no equals among enzymes of animal and plant origin was isolated from the venom of the black widow spider.

Significance and prospectivity of studies in the cotton raising taken into account, at the suggestion of Uzbekistan Academy of Sciences the activity of the laboratory was refocused on the structural-functional studies on biologically active agents. The laboratory was faced with the problem to study proteins and enzymes participating in the growth and development of the cotton.

Principle directions of research include

- Study on molecular mechanisms of the cotton phytoimmunity under effect of biotic and antibiotic factors, signaling systems determining induction of immune responses; study on the role of enzymes of pro/antioxidant system of the cotton and stress phytohormones in responses upon salinization and effects of phytopathogens; study on mechanisms of action of physiologically active natural compounds as regulators of growth and development of the cotton based on the enzymatic markers for their further use in novel biotechnologies; study on a role of phytohormones in the adaptive responses of the cotton associated with the effects with unfavorable environmental factors
- Study on mechanisms of the pro/antioxidant system and stress phytohormones of the cotton under effect of phytopathogens and salinization
- Study on mechanisms of the induced cotton and wheat resistance under effects of the glycerrhizic acid preparations isolated from the licorice roots and a supramolecular complex; elucidate principle of the prolonging activity of the low molecular weight components and enzymes of the cotton antioxidant system under stresses



- Study on the antioxidant system enzymes and phytohormones as biochemical markers of evaluation of the economically valuable features of the cotton quality varieties, subvarieties, species and lines aiming at acceleration of the novel cotton species with economically valuable features and resistant to stresses
- Development of personal recommendations for use of new generation preparations (DAG-1 and DAG-2) and chemical attractions with antioxidant status of each cotton genotype and definite edaphic-climatic conditions taken into account
- Participation in the development of the integrated personalized approach to raising of some cotton genotypes based on molecular-biochemical evaluation of pro/antioxidant statuses of the cotton upon use of up-to-date ecologically safe natural biostimulators
- Study on biological activity and mechanism of action of novel compositions based on fungicides and stimulators of natural origin.

Principle achievements include

- Complete study on mechanism of the cotton resistance to wilt induced by the *Verticilliumdahliae*and*Fusariumoxysporum*phytopathogens. Development of a test system for the accelerated determination of the wilt resistance in the cotton quality varieties, subvarieties, species and lines; the technology was testes and introduced at the Scientific-Research Institute of Selection, Seed Development and Agrotechnologyof the Cotton Raising upon novel cotton species
- Production of new generation preparations of the supramolecular complex of glycerrhizic acid and its salts (DAG-1 and DAG-2). Their stimulating and prolonging effects on the antioxidant system of the cotton resistance to phytopathogens and salinization was found
- Based on the technology for induction of systemic prolonged cotton resistance under effect of new generation preparations, there appeared the chance to increase the immune potential of the cotton resulting in the yield gain and the improvement of the cotton fiber quality. DAG-1 and DAG-2 are recommended for wide use in the cotton

farming with the aim to protect the cotton from phytopathogens and soil salinization as well as for improvement of economically valuable features of the cotton, such as improvement of cotton fiber quality, increase of a raw cotton boll mass and fiber yield. The cotton yield on the salinized soils increases by 5-8 dt/ha

- Three patents issued by the Republic of Uzbekistan Agency for Intellectual Property. To name Patent № IAP 04619 (2012) “Method for production of homogenous chitin-specific peroxidase from the cotton sprouts to be used in a test system for the wilt resistance”, Patent № IAP 05005 (2015)“Method for determination of the wilt resistance of the cotton species” and Patent № IAP 05090 (2015) “Method for control of the cotton verticilliaceous wilt”
- Toxicological-hygienic conclusion on DAG-1 and DAG-2 preparations issued by the Scientific-Research Institute of Sanitary, Hygiene and Professional Diseases, Uzbekistan Public Healthcare Ministry (2014).
- Certificates issued by the State Commission on the Facilities of Chemicalization and Plant Protection, Republic of Uzbekistan (23.01.2015, No.1A 946 and 09.02.2016, No.1A 1156) for DAG-1 and DAG-2 included in a list of “Pesticides and agrochemicals approved for wide use in the Republic of Uzbekistan”
- Complete study on responses of the cotton to the salinization and soil moisture deficit with abscisic acid, auxin and principle protective proteins at the stages of the cotton growth and development. The leading role of antioxidant enzymes and osmoprotectants in the protective function of various cotton species at the early stage of effect of abiotic factors

The findings from the studies above are the basis for further investigation of mechanisms of interaction between various systems for transfer of signals to the plant cell, biochemical processes in the plant associated with the regulation of the immunity, growth and development aiming at creation of ecologically safe methods for improvement of agricultural crops resistance.

Practical value of the results

Today, testing breeding materials requires high expenditures, time and space, and artificially made infection backgrounds. The innovation studies under way made possible introduction into practice of a method for evaluation of a breeding material based on monospecific antibodies. According to specialists, regardless of field season, due to all-year use of the accelerated test system for selection of the parent forms and lines, the time to raise novel cotton species reduces to 5-6 years versus usual 10 years. The test system will allow creation of a collective base of plant material with various technological features and high resistance to the verticilliaceous wilt jointly with the plant selection breeder. the method is used at the Scientific-Research Institute of Selection, Seed Development and Agrotechnology of the Cotton Raising making possible significant acceleration of the process for raising early ripe, highly productive cotton species characterized with high fiber quality and yield.

Based on the study of biochemical mechanism underlying effect of preparations of glycerrhizic acid from licorice roots (DAG-1 and DAG-2), they were proved to be inductors increasing the cotton resistance to phytopathogens an salinization. It was established that in infection with pathogens in the activation of various isoforms of peroxidase salicylic acid in DAG-1 connecting to the molecules of the enzyme increase the synthesis of oxygen active forms resulting in the intensification of protective responses in the cotton. Probably, the effect is associated with the prolonging activity of the phytoimmunity enzymes and actions

of DAG-1 on activation of extracellular peroxidase responsible for the cotton resistance. The activity of the preparations above was found to surpass the one of enzymes of pro-/antioxidant system responsible for stress resistance of the cotton.
According to letters from the Syrdarya region Executive, Department of Agriculture and Water Management, the Syrdarya region Center of Plant Protection, in 2017 Institute of Bioorganic Chemistry got an approval for industrial testing of DAG-1 and DAG-2 preparations on 500 ha of the salinized soils of 55 farms in the Syrdarya region. Increase of the raw cotton yield ranging from 5 to 8 dt/ha has been registered to be confirmed by the appropriate documents.

In 2018 the wide-scale tests of DAG-1 on the salinized soils of the Syrdarya region were performed; the contracts with Uzbek-British "Bek cluster" Ltd. for testing on 200 ha and with 11 farms to process the cotton on 35 ha of the salinized soils were concluded.

In 2018 in the "Bek cluster" farms, as compared with the controls the cotton yield increased by 5.7 dt/ha with the increase in the germinative capacity of the cotton seeds by 7%. In other farms the cotton yield increased by 5.6 dt/ha, in the farms of Tashkent region in the Upper - Chirchik district it increased by 4.0 dt/ha. According to the contracts, all appropriate documents were obtained from all farms to confirm the results.

The findings from the studies on molecular mechanisms of the cotton antioxidant enzymes can be used in the ecological expert evaluation of the cotton and long-term prognoses of its adaption to various stress-factors.

Prospects

- To identify the effect of DAG-1 preparation on various isoforms of the cotton peroxidase aiming at study on their molecular mechanisms upon the induced stress resistance of the cotton
- To study mechanism of the induced accumulation of prolin, malondialdehyde, prooxidants system representatives, their interrelation with salicylic acid and the cotton antioxidant enzymes upon the salinization and effects of novel natural preparations and chemical compounds
- To continue joint study with the Scientific-Research Institute of Selection, Seed Development and Agrotechnology of the Cotton Raising on the effects of the resistance inductors DAG-1 and DAG-2 on the yield and adaptive properties of various cotton species based on the markers of antioxidant system in the field conditions aiming at reduction of the rate use of domestic and foreign chemical preparations ("Baikal", EM-1", "Gummimax", "Vympel", "Albit").
- Based on the results of testing DAG-1 and DAG-2, including biochemical control of the cotton sprouts, the seeds processed with the preparations on 500 ha of the Syrdarya region (200 ha of the salinized soils in the "Bek cluster farm and 300 ha in farms, to introduce the preparations into practice with all appropriate documents, such as business plan, license contract and others completed
- Based on the instruction of Uzbekistan Cabinet of Ministers dated October 14, 2018 No.850-F, testing of personal use of DAG-1, DAG-2 and other biostimulators in

manifestation of resistance in the novel cotton species in various climatic conditions of cotton-raising regions of the Republic will be performed

- To create composition of preparations intensifying cotton immune system along with fungicides aiming at the increase in cotton seed resistance to salinization and unfavorable environmental factors.

Scientific ties

Scientific-Research Institute of Selection, Seed Development and Agrotechnology of the Cotton Raising, Uzbekistan Academy of Sciences; Center for Genomics and Bioinformatics, Uzbekistan Academy of Sciences, Gulistan State University, Higher Education Ministry.

Author's certificates and patents

1. Author's certificate on the applied sciences No.137257 (Confidential). A.S. Sadykov, Kh.A. Aslanov, A.A. Akhunov

2. Method for production of the serum from the *Latrodectus* spider venom. Authors' certificate No. 1027866, 1980, V.S. Safarova, A.S. Sadykov, U.A. Aripov, A.A. Akhunov

3. Method for production of kininase. Author's certificate No.9713490, 1982. A.S. Sadykov, A.A. Akhunov, Z. Golubenko

4. Method for identification of activity of acetylcholinesterase. Author's certificate No.3812534, 1982. A.A. Takanaev, O.N. Veshkurova, A.V. Karas, A.S. Sadykov, A.A. Akhunov

5. Method for production of the ^{125}I-labeled indicator of kinins. Author's certificate No. 4269465/31-04. 1988. A.A Akhunov, B .M. L'vov, A.A. Kolmakova

6. Method for purification of the enzyme preparations. Author's certificate №4847376. 1991. A.A. Akhunov, M.M. Rakhimov, N.A. Abdurashidova, Sh.K. Navruzov

7. Method for isolation of an inhibitor for angiotensin-1-converting enzyme from the venom of animal origin. Author's certificate No.1730088. A.A. Akhunov, Z. Golubenko, N.A. Sosnina

8. Method for predicting the cotton fiber quality. Patent No. 04370, 2000. A.A. Akhumov, Z. Golubenko, E. Mustakimova, N.A. Abdurashidova, F.A. Ibragimov, Yu.V. Beresneva

9. Method for generation of peroxidase from the cotton leaves. Patent No.AP 02862, 2005. A.A. Akhumov, Z. Golubenko, N.A. Abdurashidova, F.A. Ibragimov, G.O. Akbarova, E. Mustakimova,

10. Method for Pix and Priphonol testing on the cotton. Patent No. 03224. 31.10/2006. A.A. Akhumov, E. Mustakimova, F.A. Ibragimov

11. Method for isolation of homogenous chitin-specific peroxidase from the cotton sprouts to be used in a test system for wilt resistance. Patent No.IAP 04619 30.11.2012. A.A. Akhumov, N. Khashimova. Z.Golubenko, E. Pshenichnov, S. Vshivkov

12. Method for isolation of a protein, glucansynthase inhibitor from the cotton gymnospermous line. Patent № IAP 70100602, 17.12.2010. A.A. Akhumov, Z.Golubenko, N.A. Abdurashidova, E.Ch. Mustakimova, E. Pshenichnov, S. Vshivkov

13. Method for determination of the cotton resistance to wilt. Patent No.IAP 05005. 2015. A.A. Akhumov, N. Khashimova, E. A. Pshenichnov, V.A. Avtonomov, Sh.B. Amanturdyev

14. Method for control of cotton wilt. Patent No. IAP05090 16.09.2015. A.A. Akhumov, N.R. Khashimova, E. A. Pshenichnov, V.A. Avtonomov, D.N. Dalimov, O.D. Matchanov, M.B. Gafurov

Publications 2013-2019

1. Some Aspects of Interaction between Cotton Anionic Peroxidase and *Verticillium dahliae* Kleb. The Asian and Australian Journal of Plant Science and Biotechnology. 2013. 7 (2), A.A.Axunov, N.R. Khashimova, E.Pshenichnov, S Vshivkov, E Mustakimova, Z Golubenko, R. Stipanovic.

2. The Effect of Phytohormones on the Dynamis of Protein Biosynthesis and Enzyme Activity in Linted and Naked Cotton Seed. The Asian and Australian Journal of Plant Science and Biotechnology. 2013. 7 (2). A.A.Axunov, Z. Golubenko, E Mustakimova, N.Abdurashidova, E Pshenichnov, S Vshivkov, R Stipanovic.

3. A.A. Akhunov, Z. Golubenko, N.A. Abdurashidova, E.Ch. Mustakimova, S.O. Vshivkov, E.A. Pshenichnov Changes in concentrations of endogenous phytohormones and proteins upon effect of abiotic stresses on the cotton. Chemistry of natural compounds. 2013, No.2, 274-276

4. A.A. Akhunov, N.R. Khashimova, V.A. Avtonomov, L.A. Larin. Effect of salts of glycerrhizic acid on activity of enzymes of callus cultures of the cotton under influence of *Verticillium dahlia.* Readings from Vavilov. Samara, Russian Federation, November 2014, p.156-160.

5. A.A. Akhunov, N.R. Khashimova, V.A. Avtonomov. Proteomic technology in evaluation of resistance to the verticillium wilt of *G.hirsutum* L cotton species. Readings from Vavilov. Samara, Russian Federation, November 2014, p.161-164.

6. A.A. Akhunov, N.R. Khashimova, M.A. Mamasolieva, F. Asadov, V.A. Avtonomov, A.M. Kokhkharov. Effect of laser radiation on some physiological-biochemical processes in the cotton. Readings from Vavilov. Samara, Russian Federation, November 2014, p.165-169.

7. A.A. Akhunov, Z. Golubenko, N.A. Abdurashidova, E.Ch. Mustakimova. Analysis of function of the cotton protection system in the conditions of salinization and salt moisture deficit. Uzbek biological journal. 2014, No.6, 3-7

8. N.R. Khashimova, M.A. Mamasolieva, S.B. Navruzov, A.A. Akhunov, V.V. Uzbekov, V.A. Avtonomov. Effect of glycerrhizic-salicylic acids complex on the activity of phenyl alanin and ammonium liase, and concentrations of salicylic acid in the cotton leaves upon infection with *F. oxysporum* fungus. Readings from Vavilov. Samara, Russian Federation, November 2014, p.171-174.

9. A.A. Akhumov, N. Khashimova. D.N. Dalimov. Diglycerrhizinates, new generation preparations increasing the cotton resistance to *Verticillium dahlia.* Doklady Akademii Nauk, 2015, No.5, P.52-56.

10. A.A. Akhunov, N.A. Abdurashidova, Z. Golubenko, E.Ch. Mustakimova, N.R. Khashimova, V.A. Avtonomov. Biochemical signs of quality and wilt resistance of cotton fibers upon raising of novel species of the cotton. News of Uzbekistan Agricultural Science, 2016, No.1

11. N.R. Khashimova, M.A. Mamasolieva, M.B. Gafurov. Supramolecular complex of glyrrhezic acid with salicylic acid as an ecologically safe inductor of the cotton resistance. Uzbek chemical journal, 2016, No.4, 92-96.

12. Effect of salicylic acid on cytophysiological responses of the cotton infected with *Verticillium dahlia.* News from the National University of Uzbekistan, 2016, No.2, P. 130-133

13. N.R. Khashimova, M.A. Mamasolieva, D.T. Babaeva. Biochemical analysis of the *G .hirsutum* L cotton line resistance to the verticillium wilt. News from the National University of Uzbekistan, 2016, No.2/3, P. 146-149

14. N.R. Khashimova, A.A. Akhunov, M.A. Mamasolieva, D.T. Babaeva. Effect of salicylic acid on cytophysiological responses of the cotton infected with *Verticillium dahlia.* News from the National University of Uzbekistan, 2016, No.2/3, P. 153-157

15. Use of biochemical parameters of cotton fiber quality upon breeding of new wilt-resistant cotton species. Uzbek biological journal, 2017, No.1, P. 3-7

16. Akhunov A.A., Avtonomov V.A., Golubenko Z., Hashimova N.R., Navruzov S.B., Kuldasheva K.M. Some biochemical parameters of cotton fiber quality and wilt resistance used in selection of novel cotton cultivars. 76th Plenary Meeting of the ICAC. Digest of scientific and technical achievements. 2017, P. 28-30.

17. Z. Golubenko, A.A. Akhunov, S.B. Navruzov, G.A. Akbarova, K.M. Kuldoshova. Assessment of antioxidant status as a model for study on the cotton resistance to chloride salinization. II International Symposium. Molecular aspects of redox-metabolism of plants. June 26-30, 2017, Ufa, Russian Federation. P. 95-98

18. A. A. Akhunov, A.E. Kurbonov, N.R. Khashimova, V.A. Avtonomov Assessment of hybrid material for resistance to *Verticillium dahlia klebhan* with markers enzymes of phytoimmunity. Agricultural Science, 2019; (7-8):54-56

19. A.A.Akhunov, A.Y.Kurbonov, N.R.Hashimova, V.A. Avtonomov. Study and Identification of highly Resistant Hybrids to *VERTICILLIUM DAHLIAE*, by the enzyme peroxidase. International journal of Bio-Science and Bio-technology. ISSN: 2233-7849, V.11-Issue-8-August-2019