https://doi.org/ 10.33472/AFJBS.6.Si2.2024.2013-2023

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

EVALUATION OF HYBRID AND BREEDING MATERIAL FOR RESISTANCE TO *VERTICILLIUM DAHLIAE KLEBAN* USING MARKER ENZYMES OF PHYTOIMMUNITY

Axunov Alik Axunovich,
Doctor of Biological Sciences, Professor
Avtonomov Viktor Alexandrovich,
Doctor of Agricultural Sciences, Professor
Abdurashidova Nigora Rustamovna,
Doctor of Biological Sciences, Senior Researcher
Alimova Zarina Muzaffar kizi,
basic doctoral student
Cotton breeding, seed production and agrotechnologies research institute
Institute of Bioorganic Chemistry named after academician A.S.Sadykov of the Academy of Sciences of the Republic of Uzbekistan

Article History

Volume 6,Issue Si2, 2024

Received:27 Mar 2024

Accepted : 28 Apr 2024

doi: 10.33472/AFJBS.6.Si2.2024.2013-2023

ANNOTATION

For the first time, under the guidance of Professor A.A.Akhunov, the enzymes of fiber formation and resistance to *Verticillium dahliae Klebhan* lucansynthetase, peroxidase and cellulose were studied in order to evaluate and isolate highly resistant to *V.dahliae* in combination with high fiber quality of hybrid and breeding material created by Professor V.A. Autonomov.

Key words: cotton, breeding, hybrid, family, enzyme, resistance, *Verticillium dahliae Klebahn*, fiber, quality, selection

An important feature of peroxidase is enzymes is their exceptional lability, which allows them to react to most of the homeostasis disorders.

In the cell wall of plants, in addition to glucansynthetase, there is an enzyme cellulose, which includes B-gluconates [34].

Cotton fiber has activities, with the main one belonging to exon-(1-3)-3-d-glucanase, while (1-4)-3-4- gluconate has significantly low activity. The B-gluconate complex associated with the cotton fiber wall can also function as a trans glucosidase [10].

The methods used in biochemical studies are described in detail in the text. The determination of chitin-specific peroxidases was carried out in a sandwich version of enzyme immunoassay (ELISA).

Cotton breeding materials were included in the group resistant to *V.dahliae* if A405>2 x 405, where A405 is the optical absorption reading of the analyzed sample; and 105 is the optical absorption reading of unstable differentiator varieties.

The determination of glucansynthetase activity was determined by the cellulose content in plant material using the Underground method [10].

The protein in the samples was determined by the Lowry method [35].

Statistical processing of the research results was carried out in accordance with the methodology of O.Y. Rebrova [24].

The use of the developed test systems (Akhunov et al. 2008, Akhunov et al. 2010) allows the selection of fork-resistant samples of hybrid and breeding material, as well as using biochemical characteristics to determine the quality of fiber.

As a result of the analysis of the conducted biochemical studies, which are presented in the article, the following conclusions should be drawn:

1. The selection of a hybrid combination F_2 L-588xNamangan-102 promising in fiber quality and *V.dahliae* stability was carried out;

2. For further breeding study, as well as a starting material for hybridization, L– BSG-455-56/07 is recommended, characterized by high fiber quality and resistance to *V.dahliae.*

INTRODUCTION

For the first time in the enzyme laboratory of the Institute of Bioorganic Chemistry named after academician A.S.Sadykov of the Academy of Sciences of the Republic of Uzbekistan, under the guidance of Professor A.A.Akhunov, the enzymes of fiber formation of various lines and hybrids of cotton glucansynthetase, peroxidase, and cellulose were studied. It was found that the activity of glucansynthetase and peroxidase of pubescent lines is higher, and that it is inherited in generations [2].

An important feature of peroxidase is enzymes is their exceptional lability, which allows them to respond to most homeostasis disorders. Such lability is determined by the mechanisms of gene regulation, where there is repression or depression of a part of the genome responsible, apparently, for the synthesis of one or another is peroxidase variant.

The existing factual material supports the point of view according to which the regulation of gene activity is carried out due to a system of phytohormones acting on the genetic apparatus of the cell and its protein-synthesizing system [4].

The existence of is enzymes increases the intensity and plasticity of cellular metabolic processes in the body under stress, as well as contributes to the maintenance of the self-regulation system and expands the ways of optimization in pathogenesis [37].

Based on numerous biochemical studies, it is believed [5;38] that the peroxidase test should be used when evaluating breeding material for virus resistance.

The formation of phenolic compounds involved in redox processes is one of the characteristic features of a plant cell. The enzyme polyphenol oxidase participates in one of the stages of phenol formation [29]. Previous studies [20,25] have established that phenolic compounds are involved in the process of fiber cell nucleation. In particular, in the culture of the ovule, these compounds, for example frolic acid, stimulate the process of fiber formation [3]. It is known that the activity of this enzyme is not the same in different varieties of cotton.

The composition of plant cell walls includes cellulose, a high-molecular polysaccharide consisting of the reducing disaccharide cellobiose, in which the bond between two D-glucose residues belongs to the B-(14)-type micro fibril. According to Brown [35], celluloses can be synthesized by mobile enzyme complexes located in the thickness of the plasma lemma.

In the cell wall of plants, in addition to glucansynthetase, there is an enzyme cellulose, which includes B-gluconates. There are three classes of B-gluconates induced by various factors: environmental changes, infection by pathogenic fungi, as well as B-gluconates appearing at an early stage of seed germination [34].

Cotton fiber has activities that are in a somewhat related B-gluconate state. The main activity belongs to exon-(1-3)-3-d-glucanase, while (1-4)-3-4- gluconate has significantly low activity. The B-gluconate complex associated with the cotton fiber wall can also function as a trans glucosidase [10].

The process of enzyme biosynthesis is under genetic control. Enzyme activity is regulated by the concentration of final and intermediate products of substrate transformation, as well as environmental conditions [10].

The principal advantage of the evaluation system of the source material for breeding is the possibility of a sufficiently deep and comprehensive study of the maximum diversity of culture. Research methods play a crucial role in the effectiveness of cognition of the gene pool. In practice, this is realized in the development of methods of genetics, immunology, physiology, biochemistry, and technological assessment of fiber quality in relation to the problems of source and breeding material [11]. The methodological approaches are based on the coverage of all available genetic diversity of culture [12].

MATERIALS AND METHODS

Together with the Institute of Bioorganic Chemistry named after academician A.S.Sadykov of the Academy of Sciences of the Republic of Uzbekistan, namely with the staff of the laboratory headed by Professor A.A. Akhunov (responsible executor N.R. Abdurashidova), 11 different samples of cotton seeds presented by the breeder V.A. Autonomov were studied in order to study the activity of the main enzymes of fiber formation of glucansynthetase, cellulose and a marker of resistance to the pathogen *Verticillium dahliae Klebhan* of chitin-specific peroxidase on the presented breeding material. The provided seeds were exposed and germinated in cartridges at 27 ° C in a thermostat for 7 days. The activities were determined in the supernatants obtained from hypocotyls individually for each enzyme according to a previously worked-out scheme.

Chitin-specific peroxidases were determined in a sandwich version of enzyme immunoassay (ELISA). ELISA was performed on 96-well plates. The sequence of ELISA:

- sorption of polyclonal antibodies at a concentration of 0.2 - 0.5 micrograms/ml in 0.02 M carbonate buffer, pH 9.6 and incubation for 18 hours at 4 °;
- application of 1% BSA solution in a phosphate buffer and incubation at 37 ° for 1 hour;
- application of 100 µl of plant extract diluted in a phosphate buffer;
- introduction of a conjugate of rabbit antibodies against chitin-specific anionic peroxidases with alkaline phosphatase in working dilution and incubation at 37 ° for 1 hour;
- application of 100 µl of a substrate mixture of 0.005 Dinitrophenyl phosphate in a ethanolamine buffer, pH 10.4, with 0.002 M MgCl2, incubation for 20 minutes at room temperature;
- stopping the reaction by adding 50 µl of 5M NaOH. Between the stages, the tablets were washed 3-5 times with a phosphate buffer containing 0.05% Twin. The optical absorption was

measured on a multiplatform analyzer at 405 nm. The results were presented in the form of the optical absorption value of the resulting product of the enzymatic reaction. The group of resistant to *V.dahliae* included cotton varieties if A405>2 x 405, where A405 is the optical absorption reading of the analyzed sample; and 105 is the optical absorption reading of unstable differentiator varieties [13].

The determination of glucansynthetase activity was determined by the cellulose content in plant material using the Underground method [10].

Determination of cellulose activity in relation to Na-CMC - soluble substrate. To 40 mg of the substrate in 10 ml of 0.05 M Na-acetate buffer, pH 5.3. 0.5 ml CMC was incubated at 50 ° C on a rocking chair, 0.5 ml of an enzyme solution isolated from hypocotyls (activity 0.1-1 IU/ml by endoglucanase) was added, incubated for 20 minutes, filtered and reducing sugars were determined by the Shomadi Nelson method. To prepare the control, an enzyme solution was quickly added to the substrate suspension and reducing sugars were immediately determined. The measurements were carried out at an absorption of 610 nm. The calibration curve was based on glucose. Cellulose activity was expressed in units of activity per mg of protein [22].

The protein in the samples was determined by the Lowry method [35].

Statistical processing of the research results was carried out in accordance with the methodology of O.Y. Rebrova [24], and included testing the hypothesis of the correspondence of tabular data to the law of normal distribution using the Attestat data analysis program, v.10.9.6, working as an add-on of the Microsoft Excel-2007 program, using the modules "Checking normality" and "Emissions Treatment". Next, the calculation of the average value and the standard deviation with the help of the module "Descriptive statistics" was performed. Parametric criteria were used to calculate these indicators.

Investigation of the main enzymes of cotton fiber formation - glucansynthetases, peroxidases and celluloses – play a great practical role in the development of new varieties of cotton with high fiber quality. The use of the developed test system (Akhunov et al. 2008, Akhunov et al. 2010) allows for the selection of fork-resistant seeds, presented samples of hybrid and breeding material, as well as the study of biochemical parameters in laboratory conditions allows to determine the quality of the fiber of the breeding material.

To diagnose resistance, it is necessary to create a certain effect on the studied plants by the stress to which resistance is being investigated. In some cases, this stress load is carried out directly in the process of evaluation work, as an element of the diagnostic method [18].

RESEARCH RESULTS

In the laboratory of enzymes under the supervision of Professor A.A.Akhunov of the Institute of Bioorganic Chemistry named after academician A.S.Sadykov of the Academy of Sciences of the Republic of Uzbekistan tested the seeds of 11 samples of cotton seeds of various origins provided by the breeder of the Cotton breeding, seed production and agro technologies research institute (CBSPARI), Professor V.A. Avtonomov, in order to study the activity of the main enzymes of fiber formation of glucansynthetase, cellulose and a marker of resistance to *V.dahliae* - chitin-specific peroxidase in breeding material. For this purpose, seeds of ancestral plants, varieties, lines and hybrids of cotton used in the creation of a new variety were used in order to select breeding material with improved fiber quality, as well as highly resistant to *Verticillium dahliae Klebhan*.

At the initial stage of research, biochemical tests using marker enzymes of phytoimmunity, namely chitin-specific peroxidase, were used in the evaluation of *V.dahliae*. A sterilized strain of the *V. dahlia* pathogen isolated from the soil of the provocative background of

the Cotton breeding, seed production and agro technologies research institute (CBSPARI) was used as a stress load.

In the course of the research, the method of accelerated determination of the wilt resistance of cotton was used, for which the laboratory received a patent in 2015: "A method for determining the resistance of cotton varieties to wilt".

In order to assess the molecular genetic diversity of the source and breeding material when creating new varieties using biochemical criteria for disease resistance, parental pairs and hybrid combinations of F_2 were tested.

As a result of a biochemical study conducted in the laboratory, it was found that resistant to verticillous lesion by the pathogen *V. dahliae* should include:

- among the initial forms, the BSG 455-56/07 line;
- to a relatively stable hybrid combination F_2 L-588 x Namangan-102 (Table 1 and Figure 1).

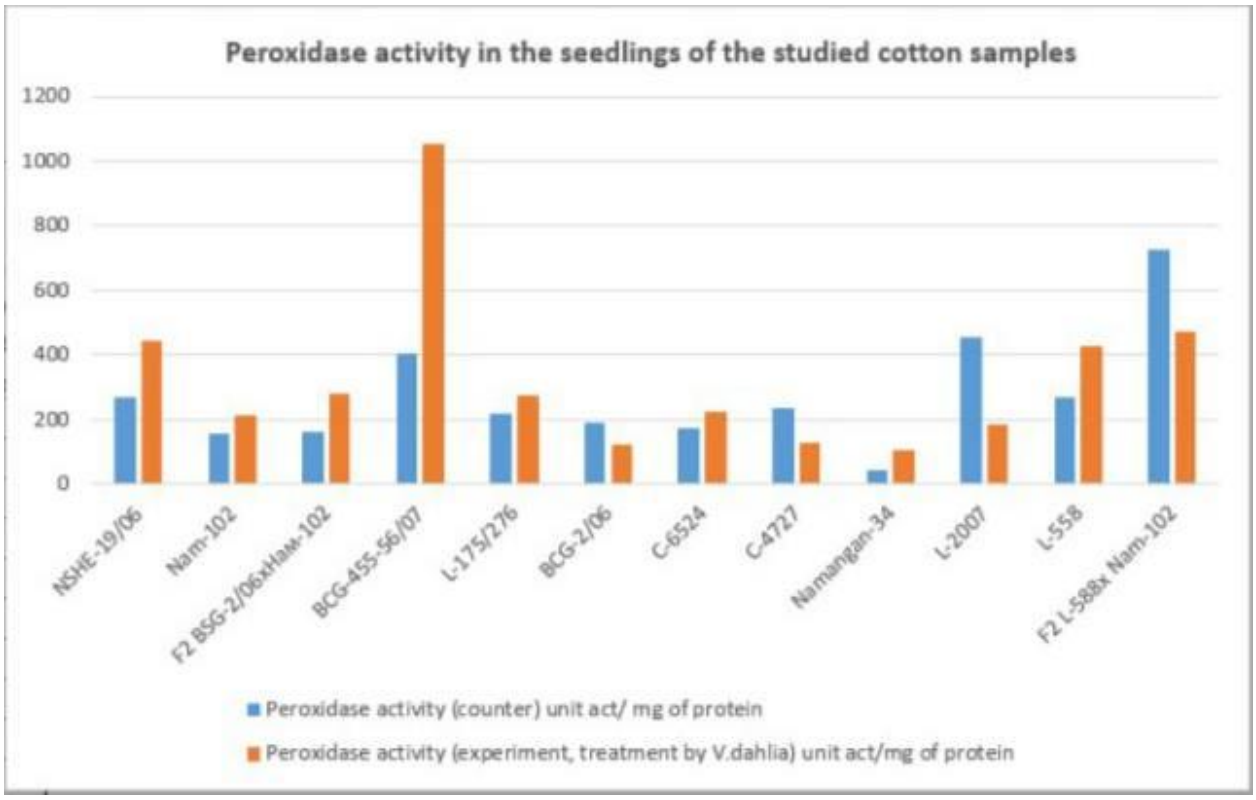
Table 1.

Peroxidase activity in the seedlings of the studied cotton samples (M±m, n=3)

№	Sample	Protein mcg/g of raw weight	Peroxidase activity (counter) unit act/ mg of protein	Peroxidase activity (experience, *V.dahlia* infection) unit act/mg of protein
1	NSHE-19/06	0,565	266,59±3,8	442,04±6,1
2	Namangan-102	0,495	153,88±0,9	210,17±1,9
3	F_2 BCG -2/06xNamangan-102	0,680	163,22±1,8	282,6±4,4
4	BCG -455-56/07	0,530	406,24±5,4	1050,80±13,7
5	L-175/276	0,653	215,81±3,5	273,50±4,0
6	BCG-2/06	0,459	190,04±2,9	120,29±3,4
7	C-6524	0,600	175,10±0,8	225,12±2,6
8	C-4727	0,530	232,40±3,9	129,49±1,7
9	Namangan-34	0,424	45,02±0,9	105,12±1,3
10	L-2007	0,565	452,80±4,3	185,70±0,6
11	L-558	0,777	266,60±5,1	427,30±3,7
12	F_2 L-588x Namangan-102	0,641	726,80±6,8	471,40±5,7

The high activity of chitin-specific peroxidase indicates the resistance of cotton to pathogenic invasion and triggers the process of phytoimmunity formation. Extracellular anionic peroxidase is known to be the main target for extracellular salicylic acid [28;29]. By attaching to the molecule of this enzyme, salicylic acid changes its catalytic activity and triggers an oxidative flare-up, a sharp increase in the synthesis of reactive oxygen species [30;31].

Fig 1



It has a destructive effect on pathogenic microorganisms and is a systemic acquired one, namely, an important secondary inducer of resistance, triggering the biosynthesis of phytoalexins, lignification of cell walls, etc. [39;40;33;34;37]. Table 2 identifies the most resistant line of BSG-455-56/07 cotton to damage by the pathogen *V. dahliae*. Of the 11 samples presented, 2 of the most stable were selected. The activity of chitin-specific peroxidase in the experimental samples exceeds the activity of the control samples by 2-16 times, which indicates that the selected samples are most resistant to wilt damage.

Table 2.

A promising cotton line for resistance to *Verticillium dahliae* (M±m, n=3)

№	Sample	Protein mcg/g of raw weight	Peroxidase activity (counter) unit act/ mg of protein	Peroxidase activity (experience) unit act/mg of protein
1	BCG-455-56/07	0,530	406,24±5,4	1050,80±13,7

In the laboratory of enzyme chemistry, the inheritance of the seed pubescence trait in parental forms used in hybridization and hybrids was previously studied. The component composition of proteins of seedlings, developing fruit elements of cotton lines of various genetic origin, differing in the degree of omission, in which enzymes of fiber formation of cotton lines were studied, while the seeds of the presented samples served as the object of study. It was found that the activity of glucansynthetase in pubescent lines with drooping leaves is higher than in gymnosperm lines; at the same time, cellulose activity is reversed and, most importantly, this feature is inherited from generation to generation [1].

The results of testing the activity and content of the cellulose enzyme in the seedlings of the presented cotton samples are shown in Table 3 and Figure 2.

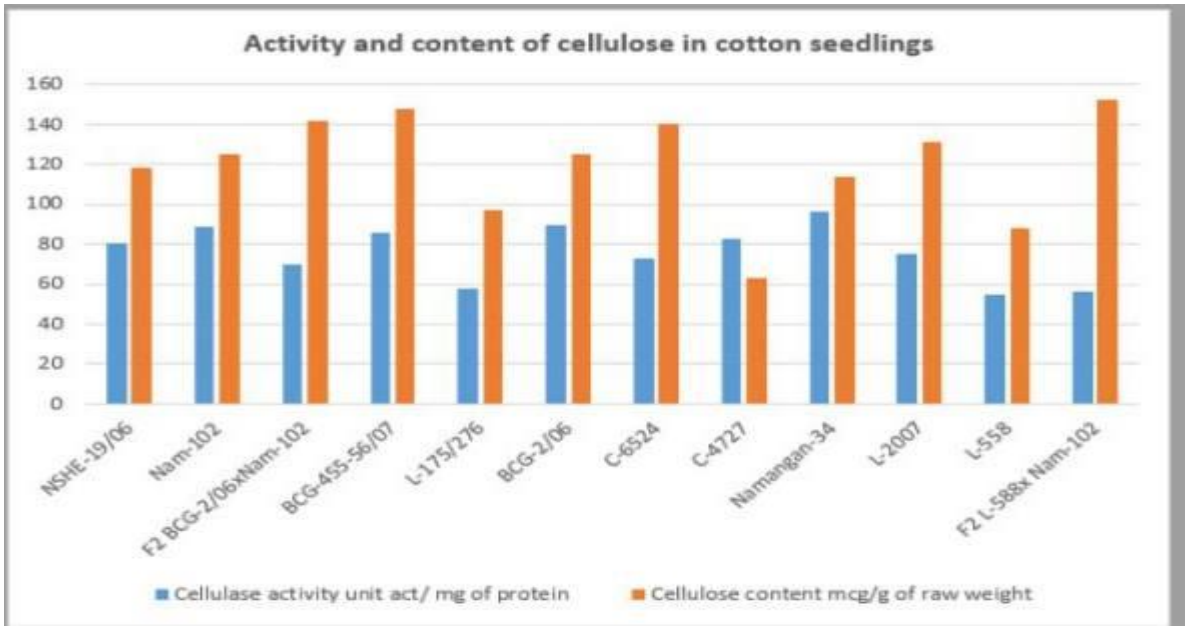
Table 3.

Activity and content of cellulose in cotton seedlings (M±m, n=3)

№	Sample	Protein mcg/g of raw weight	Cellulase activity unit act/ mg of protein	Cellulose content mcg/g of raw weight
1	Nshe-19/06	0,565	80,54±0,6	118±2,4
2	Namangan-102	0,495	88,95±0,9	125±3,6
3	F_2 BCG-2/06xNamangan-102	0,680	70,10±1,1	142±2,2
4	BCG-455-56/07	0,530	85,91±2,3	148±1,7
5	L-175/276	0,653	57,67±0,5	97±1,3
6	BCG-2/06	0,459	89,14±1,6	125±3,9
7	S-6524	0,600	73,26±1,3	140±5,7
8	S-4727	0,530	83,02±4,3	63±0,4
9	Namangan-34	0,424	96,57±1,5	114±2,9
10	L-2007	0,565	75,13±1,2	131±4,6
11	L-558	0,777	54,64±0,6	88±1,0
12	F_2 L-588xNamangan-102	0,641	56,21±0,8	152±3,5

Previously conducted experimental work has established that the enzymatic activity of glucansynthetase differs from each other in cotton samples of different origin: the higher the technological qualities of the fiber, the higher the glucansynthetase activity. The results obtained allow us to recommend the best source, hybrid and breeding material to breeders. Taking into account the biochemical parameters of the fiber, in particular, the change in enzyme activity in parent forms, hybrids and breeding material, since the resulting characteristic is genetically determined [36].

Fig 2



The results of the conducted laboratory tests

Presented in Table 3 for determining the activity and accumulation of the cellulase enzyme in the seedlings of the studied forms of cotton, as well as clearly demonstrated in the diagram (Fig. 2).

As mentioned earlier, when diagnosing a large number of breeding samples, it is advisable to apply the principle of step-by-step assessment. According to this principle, cotton samples were selected in which the accumulation of cellulose is maximal, while the activity of the hydrolytic enzyme cellulase is minimal. As a result of the conducted research, the most promising samples for the quality of cotton fiber have been identified (table. 4).

Table 4.

Promising cotton samples on the activity of fiber-forming enzymes (M±m, n=3)

№	Sample	Protein mcg/g of raw weight	Cellulase activity unit act/ mg белка	Cellulose content mcg/g of raw weight
1	F_2 BCG-2/06xNamangan-102	0,680	70,1±1,1	142±2,2
2	BCG -455-56/07	0,530	85,91±2,3	148±1,7
3	L-175/276	0,653	57,67±0,5	97±1,3
4	L-2007	0,565	75,13±1,2	131±4,6
5	L-558	0,777	54,64±0,6	88±1,0
6	F_2 L-588x Namangan-102	0,641	56,21±0,8	152±3,5

At the third stage, only promising forms of cotton selected in table 2 are evaluated for resistance to infection by the pathogen *V.dahliae* and table 4 for the quality of cotton fiber. At this stage of the assessment, the work already uses a set of several selection methods. As a result of a biochemical study conducted in the laboratory, it was proved that L-BGS-455-56/07, which is resistant to *V. dahliae* and has high fiber quality, should be attributed to L-BGS-455-56/07, which is reflected in Table 5.

Table 5.

A promising cotton line for the activity of fiber formation enzymes and resistance to *V.dahliae* (M±m, n=3)

№	Line	Peroxidase activity (counter) unit act/ mg of protein	Peroxidase activity (experience) unit act/mg of protein	Cellulase activity unit act/ mg of protein	Cellulose content mcg/g of raw weight
1	BCG-455-56/07	406,24±5,4	1050,80±13,7	85,91±2,3	148±1,7

As a result of the analysis of the conducted biochemical studies, the following conclusions should be drawn:

1. The selection of a promising fiber quality line with a high cellulose content and low cellulase activity of enzymes in cotton seedlings was carried out, a hybrid combination F_2 L-588x Namangan-102, highly resistant to *V.dahliae*, was revealed;
2. BCG-455-56/07, characterized by high fiber quality and resistance to *V.dahliae*.

REFERENCES

1. Avtonomov V.A., Ibragimov P.Sh., Egamberdiev R. The nature of inheritance of variability to black root rot and signs determining the fiber quality of intersort F1-F3 hybrids in the DIAS system // - Tashkent. - 2000.- pp.20-23.

2. Akbarova G.O. "Research of proteins responsible for pubescence of seeds of genetic lines of cotton" abstract. candidate of dissertation, Tashkent-2006.

3. Vlasova N.A., Nuritdinova F.R. Uzbek biol.zh. 1985, no.5, 42-46

4. Dmitriev A.P. Phytoalexins and their role in plant resistance. Kiev: Nauka. Dumka, 1999. 209c.

5. Egorov A.M., Osipov A.P., B. Dzantiev B., Gavrilova E.M. Theory and practice of enzyme immunoassay. M.: Higher School, 1991. 288 p.

6. Zhukovsky P.M. Cultivated plants and their relatives. // Systematics, geography, cytogenetics, ecology, origin and use. 2nd edition supplemented. – L., Kolos (Leningrad. Ed), 1964 – p.792.

7. Zdrozhevskaya S.D. Biological and toxicological substantiation of chemical means to combat root rot of thin-fiber cotton seedlings. Abstract of the dissertation. L.: VIZR, 1969.18 p.

8. Isamidinov I.T. Development of methods for predicting black root rot of fine-fiber cotton and measures to combat it. Author's abstract. dis. For the academic degree. Candidate of Agricultural Sciences. Tashkent– 1993.

9. Kim B.B. Monoclonal antibodies in the study of enzymes. // Results of science and technology. Biotechnology series. 1989. Vol. 20.

10. Konarev A.V. All-Russian Research Institute of Crop Production and its contribution to the development of agricultural science and breeding of the country. Agricultural biology. 1994, 3: 13-75.

11. Konarev A.V. The use of molecular markers in working with plant genetic resources. Agricultural Biology, 1998, 5: 3-25.

12. Konarev V.G. The principle of protein markers in genomic analysis and varietal identification of wheat

13. Kretovich V.L. Biochemistry of plants. Moscow Higher School, 1980, p. 304.

14. Lebedeva O.V., Ugarova N.N. Stationary kinetics of the hydrogen peroxide oxidation reaction in the presence of horseradish peroxidase // Biochemistry. 1997. Vol. 62. pp. 249-253

15. Maksimov I.V., Cherepanova E.A., Khairullin R.M. Chitin-specific peroxidases in plants // Biochemistry. – 2003. – Vol.68. – Issue 1. – pp.133-138.

16. Musaev D. A. et al. Genetic analysis of the signs of cotton. / Musaev D.A., Almatov A.S., Turabekov Sh., Abzalov M.F., Fathullayeva G.N., Musaeva S., Zakirov S.A., Rakhimov A.K. - Tashkent, 2005. 121 p.

17. Mustakimova E.C. The study of glucansynthetase of fiber of various varieties of cotton. Abstract of the dissertation thesis. Tashkent, 1995

18. Plechko T.N., Kirillov A.V., Ambrosova S.M., Borisova O.V., Odinets A.G. The use of monoclonal antibodies in the diagnosis of phytoviruses. Bioorganic chemistry Vol.17. No.2. pp.223-231

19. Popov P.V., Minko D.G. On the assessment of varieties for wilt resistance. // Cotton growing. Tashkent, 1974.- No. 3. - pp.33-34.

20. Popova et al. Dokl. VASHNIL, 1979, No.8, 22-25.

21. The program of breeding works until 1990 of the cotton breeding center (guidelines). Tashkent, 1980 106 p.

22. Rabinovich M.H., Savitskene R.Y., Gerasimenko V.B., et al. Bioorganic chemistry, vol.11, 10, 1985, 1330-1341.

23. Raikova N.A., Kanash M.S. In the Atlas "Structure and development of cotton" - 1937. M.-L. Ogiz Izogiz. S.157.

24. Rebrova O.Yu. Statistical analysis of medical data. Application of the STATISTICA software package Moscow, Media Sphere, 2002, 312 p.

25. Rodimtseva N.E. et al. DAN UzSSR, 1978, №7, 67-69.

26. Rubin B.A., Artsikhovskaya E.V., Aksenova V.A. Biochemistry and physiology of plant immunity. M.: Higher School. 1975. 318 p.

27. Rubin B.A., Ladygina M.E. Energy exchange and plant immunity // Plant physiology. Vol.2. M.: VINITI, 1976. p. 448.

28. Caruso, C., Chilosi, G., Caporale, C., Leonardi, L., Bertini, L., Magro, P., Buonocore, V., 1999. Induction of pathogenesis- related proteins in germinating wheat seeds infected with Fusarium culmorum. Plant Science, 140:107-120.

29. Cordier, C., Pozo, M.J., Barea, J.M., Gianinazzi, S., Gianinazzi, P.V., 1998. Cell defense response associated with localized and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Interactions, 11(10):1017-1028.

30. Godwin, B.D., Vaduvatha, S., Nair, P.M., 1996. Stabilization of phenylalanine ammonia-lyase containing Rhodotorula glutinis cells for the continuous synthesis of phenylalanine methyl ester. Enzyme and Microbial Technology, 19:421-427.

31. Gross, G.G., The biochemistry of lignification, Adv. Bot. Res., 8, 25, 1980.

32. Kawano T. Roles of the reactive oxygen species-generating peroxidase reactions in plant defens and growth induction // Plant Cell Rep.-2003. - V.21. - P.829-837.

33. Kilic-Ekici, O., Yuen, G.Y., 2004. Comparison of strains of Lysobacter enzymogenes and PGPR for induction of resistance against Bipolaris sorokiniana in tall fescue. Biological Control, 30:446-455.

34. Kumar Harsh, Kauz Kulject, Basra Amarjit S., Malik C.P. The relationship of hydrolases to fibre development in Gossypium hirsutum L. // Proc. Indian Nat. Sci. Acad. 1987. - v.53. - #3.-P.259-261.

35. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., J.Biol. Chem, 193, № 2, 265 (1951).

36. Medeghini B.P., Lorenzini G, Baroni F.R. // J. Phytopathol. 1994. V. 140. P. 319.

37. Minibaeva F.V., Gordon L.K., Kolesnikov O.P., Chasov A.V. Role of Extracellular Peroxidase in the Superoxide Production by Wheat Root Cells.// Protoplasma. 2001. V.217. P.125-128.

38. Nakov B.K., Vassilev G.N., Ivanon I.K., et al. Activity of peroxidase and its isoenzymes in virus-affected hop plants (Humulus Lupulus L.) C.r.Acad. bulg. sci. 1982. Vol. 35, N. 10. P. 1423-1425.

39. Pshenichnov E, Khashimova N, Akhunov A, Golubenko Z, Stipanovic R. Participation of Chitin-binding Peroxidase Isoforms in The Wilt Pathogenesis of Cotton. Am. J. Plant Sci. 2011. 2, 43-49

40. Siriphanich, J., Kader, A.A., 1985. Effects of CO2 on cinnamic acid 4- hydroxylase in relation to phenolic metabolism in lettuce tissue. Journal of the American Society for Horticultural Science, 110:333-335.

41. Van Esbroek G.A., Bowman D.T., Calhoun D.S., May O.L. Genetic similarity indices for ancestral cotton cultivars and their impact on genetic diversity estimates of modern cultivars.// Genetic Resources and Crop Evolution Wisconsis, 1999.