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Study And Identification Of Highly Resistant Hybrides To Verticillium Dahliae, By The Enzyme Peroxidase

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Peroxidase is the main enzyme of the phytoimmunity of cotton, which plays a great practical role in breeding new varieties of cotton. 29 samples of cotton were extracted by CBSPARI researchers and transferred for appropriate biochemical analysis to the Institute of Bioorganic Chemistry of the Academy of Science of Republic of Uzbekistan. In the experiment, the optimal conditions for the determination of peroxidase activity were determined, then its activity was revealed in seedlings of cotton seeds, and responsiveness to the most important stresses was established. In the field, the most stable families were the complex interlinear hybrids F_4: F_4[F_4(L-101 × L-108) × L-102], F_4[F_4(L-105 × L-106) × L-106], F_4[F_4 (L-105 x L-106) × L-105]. As a result of the studies, seeds of 29 different cotton samples were tested to study the activity of the enzyme chitin-specific peroxidase. For this purpose, wild-growing seedlings of species and families of hybrid cotton combinations used in the developing of a new variety have been used to select a selection material of high resistance to *Verticillium dahliae Klebhan*. In the work on evaluation for *verticillium wilt* biochemical tests using marker enzymes of phytoimmunity – "chitin specific" peroxidase were used. As a result of a biochemical study in laboratory conditions, it was established that, to the resistant pathogen *V.dahliae*, among the samples studied, one should include: the wild type of cotton *G.laxum* and the complex interlinear combination F_6[F_4(L-105 x L-108) x L- 104]. The family isolated from the F_6 hybrid [F_4 (L-105 × L-106) × L-106] was used as origin family in breeding a new cotton variety C-6577, which was transferred for examination to the State Test from 2018.

Key words: variety, enzyme, peroxidase, cotton, hybrid, wild species, resistance, verticillium wilt.

INTRODUCTION

The greatest damage to the crop, the technological properties of cotton fiber, is applied by Verticillium Wilts (Verticillium dahliae Klebhan), to which many industrial and newly created varieties quickly lose stability. According to many authors, only the creation and introduction of varieties on a new genetic basis can, to some extent, solve

the problem of increasing cotton resistance to disease. One of the ways to solve the problem of the incidence of created varieties is to attract resistance donors to the selection process, obtained with the participation of wild species (Konarev A. 1998).

Peroxidase is the main enzyme phytoimmunity of cotton, which plays a large practical role in the development of new varieties of cotton.

Peroxidase is a glycoprotein, for which carbohydrate components can be the carrier of the corresponding variability. The main function of cell wall peroxidase is to participate in the process of cell growth and differentiation. This enzyme accelerates the hydroxylation of proline, which is part of the cell walls, which affects the stretchability and, as a result, the permeability of cell membranes is regulated. Peroxidase substrate is phenolic compounds that participate in the formation of the primary cell wall of cotton fiber.

Recent studies have shown that plant peroxidase has a certain affinity for polysaccharides, including those that make up the cellular integuments of the mycelium of fungi. For example, the ability to bind some plant apoplastic peroxidases with pectins (Nurenberger T. Et al., 1999; Zheng et al., 2004; Ivachenko 2011) and chitin (anionic in cereals) (Maximov I. et al. 2003). Works RM Khairullin (Khairullin R. 2000) showed that the anionic isoenzymes of peroxidase roots and coleoptile of wheat seedlings are directly associated with chitin under conditions that do not inactivate the enzyme. Based on the results obtained, the authors suggest possible mechanisms for the participation of wheat peroxidase anions in defense reactions when infected with phytopathogenic fungi.

Based on numerous biochemical studies, it is believed (Egorov A. et al. 1991; Nakov B. Et al., 1982) that the peroxidase test can be used in evaluating breeding material for resistance to viruses. H.F. Navar and others (Navar H. Et al., 2003) believe that the stress-induced anionic peroxidases they studied can be used as a marker to assess the resistance of legumes to the fungus B.fabae.

A characteristic feature of enzymes including peroxidase is the specificity of the action. Each enzyme acts on a strictly defined substrate. The activity of enzymes in the cell is strictly regulated. The process of enzyme biosynthesis is under genetic control. The activity of enzymes is regulated by the concentration of the final and intermediate products of the transformation of the substrate, as well as environmental conditions [Konarev A. 1994].

In the experiment, the optimal conditions for determining its activity were determined, then its activity was found in seedlings of cotton seeds, and responsiveness to the most important stresses was established.

The low specific activity of cultural cotton enzymes is compensated by an increase in the number of their forms, which increases the resistance of plants in various growing conditions. As a result of the experimental work, the influence of the phytopathogen on the change in the enzymatic activity of seedlings of cotton seeds of various phylogenetic and ecological-geographical origin was established.

Materials and methods

In the field experience of 2012, there were 2 varieties of indicators (ind) C-6524, C-4727 sown annually in an area of more than 200 thousand hectares and families of the best breeding-significant 9 hybrid combinations F_4. Resistance to *Verticillium dahliae* Klebhan determined by six points system developed (Popov, Minko., 1974).

Laboratory studies were carried out in 2015-2016, at the Institute of Biological Chemistry named after Academician A.Sadikov.

From the Cotton Breeding, Seed Production and agrotechnologies research institute (CBSPARI), prof. VA Avtonomov presented 9 samples to study the activity of the main enzymes of the marker of resistance to pathogens of chitin-specific peroxidase in the original and breeding material.

The determination of chitin-specific peroxidase was carried out according to the method of N.Hashimova.

Statistical processing of the material was carried out in accordance with the method of O. Rebrova and included testing the hypothesis about the compliance of tabular data with the law of normal distribution by means of the AtteStat v.10.9.6, data analysis program, then, the mean value and the standard deviation σ were calculated using the "Descriptive Statistics" module. When calculating these indicators, parametric criteria were used.

RESULTS

The hybrid combinations participating in the experiment were created earlier by the method of complex, interlinear hybridization, which are characterized by ultra fast ripeness and high yields raw cotton and signs that determine the quantity and quality of fiber. Hybrids were sown on natural natural background in field conditions, along with varieties acting

as indicators (ind) - C-6524 and C-4727. The values of the "defeat of *V.dahliae* plants at 1.09.12 y.", As can be seen from table 1, were placed in variational series, ranging from 0 to 5 points, with the F4 hybrids families being the most stable: F4 [F4 (L- 101 × L-108) × L-102], F4 [F4 (L-105 × L-106) × L-106], F4 [F4 (L-105xL-106) × L-105], in which the mean value the trait ranged from 0.77 to 0.79 points.

Table 1. Variability of the sign "defeat of *V.dahliae* plants at 1.09.12 g." In complex, interlinear hybrid combinations F_4 of cotton of the species *G.hirsutum L*

№	Variety and hybrid combination	K=1 point						M±m (point)	δ	V%
		0	1	2	3	4	5			
1	C-6524 (ind)				124	95	83	4.26±0.05	0.83	19.41
2	C-4727(ind)				76	108	138	4.28±0.03	0.59	13.89
3	F_4[F_4(ЛІ-101 × ЛІ-108)×ЛІ-106]	46	7	5	1	1		2,78±0,11	0,91	32,62
4	F_4[F_4(ЛІ-101 × ЛІ-108)×ЛІ-105]			1	2			3,07±0,31	0,54	17,75
5	F_4[F_4(ЛІ-101 × ЛІ-108)×ЛІ-102]	39	3	2	4			**0,79±0,14**	0,99	124,1
6	F_4[F_4(ЛІ-101 × ЛІ-108)×ЛІ-101]	16	14	3	3			1,21±0,20	1,21	100
8	F_4[F_4(ЛІ-105 × ЛІ-106)×ЛІ-106]	57	3	2	1			**0,56±0,07**	0,56	100,8
9	F_4[F_4(ЛІ-105 × ЛІ-106)×ЛІ-105]	292	36	27	12	3		**0,77±0,05**	0,9	116,5
7	F_4[F_4(ЛІ-103 × ЛІ-106)×ЛІ-105]	74	23	16				1,20±0,12	1,34	111,5
8	F_4[F_4(ЛІ-103 × ЛІ-106)×ЛІ-102]	108	31	25	16		6	1,22±0,10	1,41	115,4
9	F_4[F_4(ЛІ-104 × ЛІ-108)×ЛІ-106]	14	12	6	4		3	3,61±0,48	3,02	83,89

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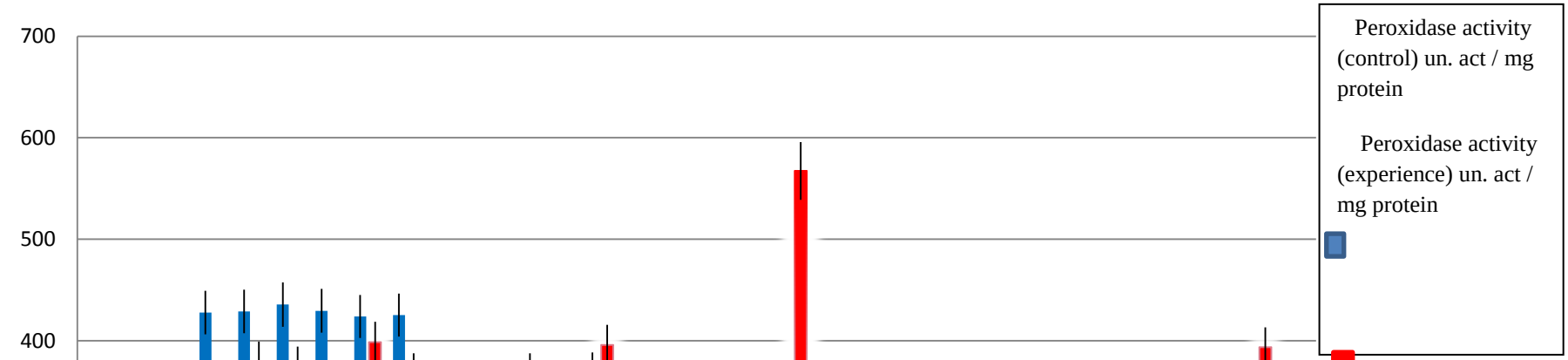
Table 2. The activity of peroxidase in seedlings of the investigated samples of cotton



Peroxidase activity in seedlings of the samples studied cotton. Then, 29 samples, of which 8 wild-growing forms of various ecological-geographical origin and 21 complex interlinear hybrid combinations of cotton, were studied under laboratory conditions, in order to study the activity of the main enzymes of the pathogen resistance chitin-specific peroxidase marker. As a result of the experimental work, a change in the enzymatic activity of seedlings of cotton seeds of various phylogenetic and ecological-geographical origin was revealed. Analyzed and summarized the patterns of biochemical adaptation of wild and cultivated cotton to growing conditions. and studied the relationship of the diversity of enzyme forms, depending on the origin of cotton samples used in the experiment. A method for using enzymatic activity as a marker of resistance of cotton samples to various agro-ecological conditions is proposed. Identified forms of cotton that should be used in the introduction and adaptive selection in order to increase the adaptive capacity of cotton.

Use of the developed under the guidance of prof. Akhunova A.A. test system allows selection of wilt-resistant specimens. Therefore, the study of biochemical parameters allows in the laboratory to determine the stability of the plant material of cotton.

As a result of the research conducted, seeds of 29 different cotton samples were tested to study the enzyme chitin-specific peroxidase.

For this purpose, seedlings of wild-growing species and families of hybrid combinations of cotton are used, used to create a new variety, in order to select a breeding material with improved properties of cotton fiber with high resistance to Verticillium dahliae Klebhan.

In our work, when evaluating vital stability, we used phytoimmunity marker enzymes — chitin-specific peroxidase — for the assessment of vital stability.

As a result of biochemical research in the laboratory, it was established that among the studied samples, resistant to damage by the V. dahliae pathogen include: wild-growing species of *G.laxum* cotton and gibrid F_6[F4 (L-105 x L-108) x L-104] (Table 1).

The results obtained when testing the activity of the enzyme peroxidase in the control (without infected) and infected with pathogens *V.dahliae* cotton seedlings became the basis for concluding about the high resistance of the above samples to V. dahliae. Under biotic stress, the defense mechanism of the trait is hidden in optimal conditions and is realized only when cotton plants are influenced by an extreme factor.

In the cotton samples noted by us, there is an increase in the activity of this enzyme by several times compared with the control, which indicates the inclusion of a systemic resistance mechanism.

CONCLUSIONS

As a result of the analysis of the research we can say that:

- in field conditions, complex interline hybrids F_4 [F4 (L-101 × L-108) × L-102], F_4 [F4 (L-105 × L-106) × L-106], F_4 [F4 (L-105xL -106) × L-105], showed high resistance, while the average value of the sign "plant susceptibility to V.dahliae, at 1.09.12" was respectively equal to 0.79, 0.56, 0.77 points;

- the family isolated from the hybrid F_6[F4(L-105×L-106)×L-106] is used as the ancestral family in the development of a new variety of cotton C-6577, which is transferred to the study of the competitive test in 2018;

- by conducting a biochemical study in the laboratory found that resistant to the pathogen V. dahliae was a complex interline hybrid combination of F6[F4(L-105 x L-108) × L-104], and among the 8 wild cotton species involved in the experiment G. laxum and showed high resistance;

- one of the most promising wild species is G. laxum, which can be used in the future as a genetic material for the development of new varieties of cotton resistant to wilt.

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