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Studying the activity of nucleic acid synthesis in cotton genotypes with different degrees of resistance to negative environmental factors

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Abstract

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The existence of a robust gene pool, encompassing varieties or accessions resistant to both abiotic and biotic environmental factors, stands as a crucial determinant for the development of cotton cultivation. At the Genetic Resources Institute of the Ministry of Science and Education of Azerbaijan, the cotton collection encompasses approximately 1500 accessions. The main objective of the study was to conduct a comprehensive assessment of drought resistance and wilt sensitivity within the cotton collection and to compare nucleic acid synthesis in genotypes exhibiting diverse degrees of drought resistance.As a result of the assessment of drought resistance the cotton accession were divided into groups with varying degrees of resistance. Genotypes characterized by resistance to both abiotic and biotic environmental factors have been identified. A correlation has been observed between the drought sensitivity and the activity of nucleic acid synthesis. In drought-resistant accessions, stress triggers the activation of DNA and RNA synthesis, indicating an increase in the physiological labilityand enhancement of functional activity of the genetic apparatus. Conversely, in stress-sensitive genotypes a reduction in nucleic acid synthesis was observed. The degree of stress resistance represents the inherited potential ability of the organism to adapt and is realized under the influence of an extreme factor.

Keywords: cotton; abiotic and biotic stresses; resistance; nucleic acids

Introduction

Cotton, belonging to the genus *Gossypium* L. (which, in Latin, means “a tree that gives fiber”) of the family Malvaceae Juss., encompasses 35 species, as per the current classification by F.M. Mauer. Among these, five are widely cultivated: *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L., *G. herbaceum* L., and *G. tricuspidatum* L. *G. hirsutum* L., native to Central America (Mexico), is the most prevalent in cultivation. On the other hand, *G. barbadense* L., originating from South America (Peru), is less common in cultivation, primarily due to its later maturation (Wendel and Grover, 2015HYPERLINK https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorRaw=Xu%2C+Li).

Cotton was introduced to Azerbaijan from neighboring Iran, where, according to certain historical documents, its cultivation dates back as early as the 11th century BC. Initially, cotton cultivation in Azerbaijan progressed modestly and relied solely on local cotton varieties known as "guzes" (referred to locally as "kara-koza," *G. herbaceum* L.). These guz varieties were low productive with short, coarse fibers that failed to meet the standards of the textile industry. Subsequently, the old local varieties were replaced by more productive and higher-quality cotton varieties of the *G. hirsutum* L. species. In certain regions with the warmest and longest growing seasons, fine-staple cotton varieties of the *G. barbadense* L. species are also cultivated.

Azerbaijan is situated in regions characterized by high summer temperatures and low relative humidity. Across most of the territory of the country, the maximum summer temperature frequently surpasses 40°C, and the relative humidity ranges between 67-71%. Drought exacerbates the nutritional conditions of plants, resulting in a deceleration of cotton development, alterations in the quality of raw cotton, a reduction in fiber length and strength, ultimately leading to reduced plant productivity. A substantial decline in cotton yields is also due to various diseases that manifest at different phases of plant development. Particularly harmful are wilt (verticillium and fusarium wilt), root rot, gummosis, macrosporiosis, powdery mildew, as well as boll and fiber diseases (Amanda et al., 2015; Sudip et al., 2020). The extent of plant resistance to stress varies both among different species and within different varieties of the same species (Lizana et al., 2006; Mammadova et al., 2016).

At the Genetic Resources Institute of the Ministry of Science and Education of Azerbaijan, which coordinates the collection, conservation, and study of various plants (Aliyev and Akparov, 2002), the cotton collection comprises approximately 1500 accessions. The existence of a robust gene pool, encompassing varieties or accessions resistant to abiotic and biotic environmental factors, stands as a crucial element in the successful development of cotton cultivation.

The lever for implementing adaptive rearrangements is the metabolic coordination system, ultimately controlled by nuclear DNA gene regulation. This control is fulfilled through the activity of enzymatic systems and is limited by the energy potential of the cell and the organism as a whole. It was confirmed that resistance to abiotic is a multigenic trait (Kotak et al., 2007). In unfavorable situations, the rapid response of plants manifests through gene expression (Abid et al., 2017). The study of the expression of defense response genes in cotton revealed a more pronounced and faster regulation in the resistant variety (Cui et al., 2000; Xu et al., 2011; Naeem et al., 2021).

Given the abovementioned, the objective of this study was i) to assess the resistance to abiotic and biotic environmental factors in cotton accessions belonging to *G. barbadense* L. and ii) to conduct a comparative analysis of nucleic acid synthesis in resistant and drought-sensitive genotypes. The study of nucleic acid synthesis activity holds significant importance in elucidating the mechanisms of adaptation of plant organisms to stress.

Materials and Methods

Seventy cotton accessions of *G. barbadense* L. were used as a research material.

Evaluation of stress-resistance to drought by seed germination in an osmotic solution

Cotton seeds were germinated in sucrose solutions, which simulates physiological drought with an osmotic pressure of 7 atm (Aliyev and Mamedova, 2007). Despite the uniform abiotic stress impact level, the extent of decrease in seed germination varied among the samples. The percentage of germinated seeds (P) is determined by taking the average number of germinated seeds per cup in the control as 100%, with the average number of seeds germinated in the experiment (a) expressed as a percentage of the number of seeds germinated in the control (b). Thus,

$$P = \frac{a}{b} \times 100\%.$$

The average number of germinated seeds in the control is taken as 100% (x), and the average number of germinated seeds in the experiment is taken as (y). The degree of depression (Z) is determined by the formula:

$$Z = 100 - \frac{x}{y} \times 100\%.$$

Assessment of stress-resistance to wilt

Wilt disease manifests as the appearance of yellowish, round, or angular spots on the leaves of the lower tier of the bush. These spots are randomly scattered across the leaf blade. To evaluate the resistance of cotton varieties of *G. barbadense* L. to verticillium wilt in Absheron condition a comparative phytopathological assessment was carried out using a five-point scale (Table 1) (Voitenok, 1970).

Cluster analysis of the degree of stress resistance of the studied genotypes was performed using the UPGMA method of the SPSS 16.0 program.

Table 1. Scale of indicators of resistance of cotton varieties to wilt

Degree of resistance	Resistance, %	Resistance, five-point scale
Immune	0	0
Highly resistant	1 – 10	1
Resistant	11 – 25	2
Tolerant	26 – 50	3
Susceptible	51 – 80	4
Highly susceptible	81 – 100	5

Determination of the content of nucleic acids

The quantification of DNA and RNA content was executed as follows: 0.1–0.2 g of dry matter was homogenized with 10 ml of cold 95% ethanol, and subsequently, the homogenate was transferred into centrifuge tubes containing an additional 10 ml of cold ethanol and centrifuged. The supernatant was then discarded. The resulting precipitate underwent washing with 20 ml of each of the following reagents:

- 95% ethanol – once at room temperature;
- 50% ethanol acidified with glacial acetic acid at pH 4.5 – twice at room temperature;
- 0.2n $HClO_4$ – twice, conducted in a cold environment;
- 95% ethanol – once at room temperature;
- Absolute ethanol with ether (3:1) – applied for 3 min, twice;
- Ether at room temperature – once.

The precipitate was subsequently dried in a vacuum desiccator and weighed. The pretreated material was then suspended in 5 ml of 0.3N NaOH and incubated at 30°C for 16 h. Following incubation, the hydrolyzate underwent centrifugation, and the resulting precipitate was washed with 5 ml of 0.3N NaOH. The extract was adjusted to a 10 ml volume with 0.3N NaOH, acidified to pH 1 with 15% $HClO_4$, kept at +4°C for 40 min, and then centrifuged. RNA was in the supernatant, while the DNA-protein complex remained in the sediment. The sedimented precipitate was resuspended in 2 ml of water, followed by the addition of 2 ml of 1N $HClO_4$. This mixture was kept at +4°C for 20 min, centrifuged, and the resulting centrifugate was combined with the RNA fraction. The total volume of the extract was adjusted to 25 ml with water.

The DNA precipitate-protein complex was suspended in 3 ml of 0.5N $HClO_4$, heated in a water bath at 70°C for 15 min, cooled, and then centrifuged at +4°C. The protein precipitate was washed with 2 ml of cold 0.5N $HClO_4$, centrifuged, and the washing centrifugate was combined with the main extract. The total volume of the extract was adjusted to 5 ml with a 0.5N $HClO_4$ solution.

DNA preparations were measured against 0.5N $HClO_4$, and RNA was measured against water. The measurements were conducted at wavelengths of 270 and 290 nm, and the calculations were performed using the following formulas:

$$\text{RNAmg/ml} = \frac{E270 - E290}{0.19n} \times 10.5$$

$$\text{DNAmg/ml} = \frac{E270 - E290}{0.19n} \times 10.1,$$

where E is the optical density;

n is the sample size in mg;

0.19 – empirically derived constant coefficient;

10.5 – constant coefficient for RNA;

10.1 is a constant factor for DNA.

The obtained results from the analyses were statistically processed (Dospechov, 1985).

Results and Discussion

Cotton is notably susceptible to the influence of unfavorable environmental factors, making it less resistant to stress, particularly during the germination stage (Akparov et al., 2006; Andrea et al., HYPERLINK "https://pubmed.ncbi.nlm.nih.gov/?term=Dever%20JK%5BAuthor%5D"2021). Identifying the traits that determine a form or variety's resistance to adverse environmental conditions involves considering the physiological characteristics of the plant (Tiago et al., 2022). The physiological response to stress entails an emergency mobilization of the adaptive potential, facilitating a temporary coping mechanism against adverse effects and, thus, holding adaptive value. Given that variations in the mechanisms of perceiving and transducing stress signals in plants contribute to different stress tolerances, studying the stress response enables the revelation of the comparative degree of resistance among plants against the impact of abiotic environmental factors.

This section of the study presents the results of evaluating the stress response to drought in a cotton collection. The stress response, facilitating the transition of the plant from normal to stress conditions, aims to initiate the development of specialized or long-term resistance mechanisms, thereby enhancing the organism's viability under altered conditions.

As indicated by the study results, the influence of the stress factor was not uniform across different cotton varieties due to genetic specificity. Varieties of the same species exhibited significant differences in seed germination amplitudes under stress conditions, depending on the genotype. The response of various variety samples to drought allowed for the conditional categorization of these samples into

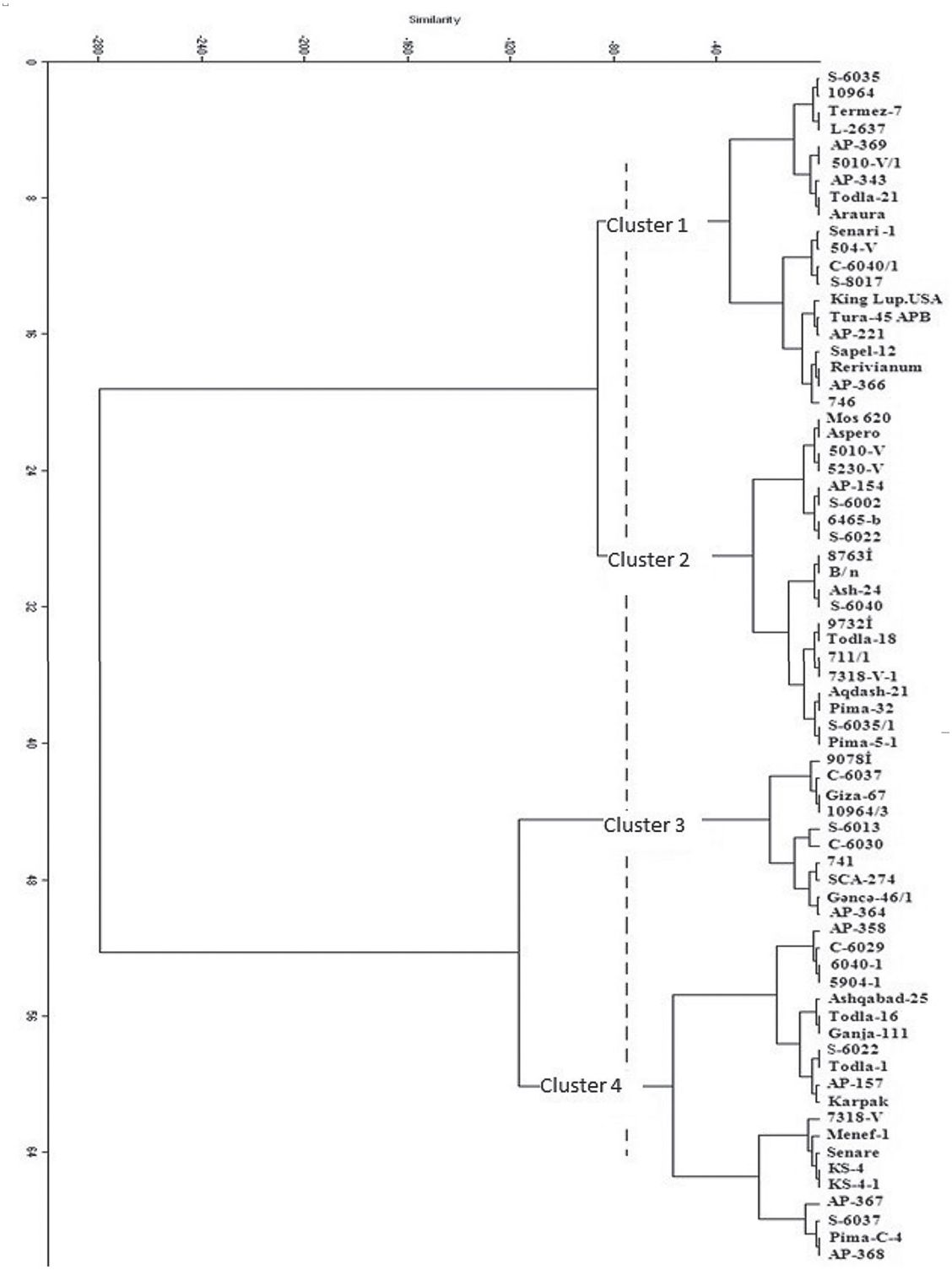

Fig. 1. Grouping of cotton accessions according to their drought resistance degree

groups with varying degrees of resistance. Cluster analysis grouped accessions into 4 clusters according to their stress resistance (Figure 1).

Samples of cotton Mos-620, Aspero, 5010-V, 5230-V, AP-154, S-6002, 6465-b, S-6022, 732I, Todla-18, 711/1, 7318-V-1, Agdash -21, Pima-32, S-6035/1, Pima-5-1, 8763I, B/n, Ash-24, S-6040, have been identified as highly resistant to drought stress, and they were clustered together in the second cluster.

The ability of seeds from these drought-resistant accessions to germinate under stress reflects, on one hand, their inherent ability to germinate with a relatively limited amount of water and, on the other hand, the presence of a high suction power enabling rapid water absorption. This high suction power not only leads to better germination in the absence of sufficient moisture but also results in the development of a more robust primary root system, crucial for the plants' survival during drought conditions.

In subsequent studies, this collection material underwent field evaluation for disease resistance, with wilt-resistant cotton samples being identified. Table 2 displays cotton accessions exhibiting high resistance to a combination of adverse factors, including drought and wilting.

The impact of environmental stress factors can induce various structural and functional changes, initially aimed at ensuring the organism's survival. As the genetic apparatus plays a significant role in coordinating these changes, determining the types of stress proteins, their intensity, and the sequence of synthesis by the cell in a given situation, we studied the nature of nucleic acid synthesis in drought-resistant and drought-sensitive cotton genotypes.

Table 2 presents the results of a study of the activity of nucleic acid synthesis during drought stress in stress-resistant cotton varieties determined according to the seed germination indicators in sucrose solution. The findings indicate that these genotypes exhibit an overall increase in the amount of DNA under stress when compared to control plants. The maximum increase in DNA was observed in the S-6002 variety, reaching 11.9%. Thus, in drought-tolerant cotton accessions, stress triggers the activation of DNA synthesis.

Regarding RNA, it is noteworthy that under drought conditions, the synthesis activity in experimental plants of drought-resistant cotton varieties 9732I, 5010-V, and S-6022 exceeds that of control plants by 10.0%, 12.3%, and 35.6%, respectively. Drought-resistant accessions AP-154 and S-6002, under stress conditions, also surpass the activity of RNA synthesis in control plants by 34.6% and 17.8%, respectively.

The study of the quantitative content of nucleic acids under stress revealed a decrease in the synthesis of both DNA and RNA compared to the control (Table 3). A com-

Table 2. Cotton accessions characterized by resistance to abiotic and biotic stresses

Cotton accessions	Drought resistance indicators			Wilt resistance index		
	Seed germination, %			%	point	The degree of resistance
	Control	NaCl	In % ofcontrol			
Mos-620	80.0	80.0	100	12.5	2	resistant
Aspero	78.8	78.8	100	0	0	immune
5010-V	100	100	100	0	0	immune
5230-V	86.8	86.8	100	0	0	immune
AP-154	80.0	80.0	100	10.0	1	highly resistant
S-6002	92.0	92.0	100	0	0	immune
6465-b	85.0	85.0	100	17.6	2	resistant
S-6022	95.0	95.0	100	8.3	1	highly resistant
9732İ	92.0	92.0	100	0	0	immune
Todla-18	90.0	90.0	100	0	0	immune
711/1	85.2	85.2	100	0	0	immune
7318-V-1	78.8	78.8	100	7.2	1	highly resistant
Aqdash-21	96.0	96.0	100	0	0	immune
Pima-32	80.0	800	100	16.7	2	resistant
S-6035/1	95.0	95.0	100	20.0	2	resistant
Pima-5-1	80.0	80.0	100	0	0	immune
8763İ	80.0	80.0	100	20.0	2	resistant
B/n	80.0	80.0	100	0	0	immune
Ash-24	78.8	78.8	100	0	0	immune
S-6040	72.0	72.0	100	0	0	immune

Table 3. Changes in seed germination, DNA and RNA content in cotton genotypes under drought conditions

№	Varieties	Seed germination during drought, in %				DNA, mg%		RNA, mg%	
		Control	Expe-riment	In % relative to control	Stress-depression, %	Control	Experiment	Control	Experiment
	Stress-resistant cotton varieties								
1.	AP-154	76.0	76.0	100	0	12.71±0.07	13.70±0.05	105.0±4.3	141.31±2.9
2	9732I	92.0	92.0	100	0	14.56±0.09	15.47±0.08	117.9±2.4	129.72±1.6
3.	5010-V	100	100	100	0	14.56±0.08	15.73±0.07	107.6±5.2	120.89±1.3
4.	S-6022	95.0	95.0	100	0	16.49±0.08	17.60±0.07	123.8±1.5	167.88±2.6
5.	S-6002	92.0	92.0	100	0	13.42±0.05	15.01±0.05	109.5±2.6	128.98±1.4
Stress sensitive cotton varieties									
1.	C-6040	89.2	76.0	85.2	14.8	16.81±0.08	15.49±0.05	125.3±2.6	111.87±3.4
2	5904-1	78.5	50.0	63.7	36.3	15.53±0.05	14.97±0.04	120.2±1.4	103.22±1.8
3.	Senare	94.8	40.0	42.2	57.8	16.10±0.07	15.44±0.07	122.4±2.4	107.46±2.6
4.	741	80.0	20.0	25.0	75.0	14.05±0.07	12.72±0.08	112.4±1.6	72.86±1.7

parative analysis of accessions with varying degrees of sensitivity to drought showed that the most significant decrease in DNA synthesis is observed in cotton variety 741, where the physiological indicator experiences the highest stress depression among the studied samples, reaching 75%. The decrease in DNA synthesis in this accession was 10.5%.

In stress-sensitive cotton accessions, a decrease in RNA synthesis was noted (Table 3). For instance, in the Senare cotton variety, the decrease in RNA synthesis compared to control plants was 12.2%, and in the 5904-1 variety, it was 14.1%. Notably, the greater the depression of a physiological parameter under stress conditions, the more pronounced the reduction in RNA synthesis. To illustrate, the C-6040 variety exhibited the lowest depression in the physiological indicator under stress (14.8%), accompanied by a 10.7% reduction in RNA synthesis. Conversely, in cotton variety 741, characterized by the highest degree of suppression of seed germination under drought stress (75%), a significant decrease in RNA synthesis was also observed (35%).

Conclusions

Hence, it has been established that under the same conditions of stress exposure, different cotton varieties exhibit significant variations in the amplitude of changes in the physiological parameter. This variability facilitated the categorization of genotypes into clusters with distinct degrees of resistance. The amplitude of physiological indicators under stress correlates with the level of plant resistance, representing the organism's inherent potential for adaptation, which is realized under the influence of extreme factors.

A comprehensive assessment of the degree of resistance has enabled the identification of cotton samples characterized by resilience to both abiotic and biotic environmental factors. The greater resistance of cotton cultivars to stress determines their capacity to maintain a normal level of metabolism across a broader spectrum of unfavorable stress factors and their ability to rapidly instigate protective metabolic changes.

The activation of DNA and RNA synthesis in stress-resistant genotypes indicates an increase in physiological lability and functional activity of the genetic apparatus. Unstable plants, influenced by negative environmental factors, tend to be more conservative, and a reduction in nucleic acid synthesis in these plants indicates such conservatism. The depression observed in the stress-sensitive samples is attributed to alterations in the structural state of total DNA, transitioning from a loosened (labile DNA) to a less active state densely packed with histones (stable DNA). In this stabilized structural state, the functional activity of DNA is noticeably reduced, particularly in its role as a general regulator of synthetic metabolic reactions, leading to a discoordination between synthesis and hydrolysis products within the cell. These changes in stress-sensitive plants result in a reduction in the intensity of synthetic processes. The intensity of growth processes, depending on the level of synthetic reactions and integrally reflecting it, decreases in plants under stress conditions, which ultimately leads to a decrease in productivity. The varying degrees of resistance observed in different varieties of the same species under identical stress exposures align with the established connection between the degree of sensitivity to abiotic stress and DNA and RNA synthesis.

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