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Review Article

Adaptive Response of Cotton to Salinity Stress

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Abstract

The article presents the results of studying the adaptive response of collection varieties of cotton of the species G.barbadense L. to salt stress. The degree of resistance of collection cotton accessions to salt stress was studied based on the indices of seed germination in an osmotic solution. The studies have shown that varieties of the same species differed significantly in the amplitude of the physiological parameter. The reaction of the samples to the action of an unfavorable environmental factor made it possible to conditionally divide the samples into groups within the species, determining different degrees of comparative resistance. The nature of nucleic acid synthesis, including the fractional composition of DNA, was studied in cotton accessions characterized by different degrees of stress resistance. The study showed that in salt-tolerant accessions, stress causes activation of RNA synthesis and total, labile, residual DNA fractions, a decrease in the amount of stable DNA, which indicates an increase in physiological lability and functional activity of the genetic apparatus. In stress-sensitive varieties, a decrease in RNA and DNA synthesis is observed, and the ratio of DNA fractions shifts towards stability. The obtained data indicate the influence of stress factors on the structure and functional activity of the plant genome.

Key words: stress; salinity; cotton; degree of resistance; rna; dna fractions

Introduction

The action of environmental stress factors can cause numerous structural and functional changes that are initially aimed at ensuring the survival of the organism. Among these changes, the reaction of the genetic apparatus plays a significant role, on which largely depends what proteins, with what intensity and sequence will be synthesized by the cell in the current situation. [Kuhlemeier and Green, 1987; Kuznetsov and Shevyakova, 2006). Processes occurring at the level of transcription and translation quickly respond to stressful environmental conditions (Blekhman and Shelamova, 1992; Bhoite et al., 2025). The reaction of the genetic apparatus to changes in environmental conditions first became known in the early 1960s thanks to the research of the Italian geneticist Rittosa (Rittosa, 1962). There are reports in the literature on the nature of changes in the genetic material of plants under the influence of abiotic environmental factors (Munns, 2005; Aroca et al., 2006; Chhapekar and Gorakshnath, 2024). Of particular interest is the potential of the eukaryotic genome, which, as is known, is in a highly repressed state at any period of ontogenesis. Mass "reading" of information occurs simultaneously on a very small part of genes, the composition of which differs in different types of tissues (Kulaeva, 1988). When environmental conditions change, a program of selective gene expression is implemented and mechanisms of genetic determination of plant resistance to stress are carried out. Such a program is implemented in the system of a whole cell. The first studies of gene expression, which is a rapid response of the organism, in higher plants date back to the mid-70s of the 20th century. Nagle (Nagl, 1976) discovered that the transition of genes from an inactive state to an active one and vice versa depends on a wide variety of factors – photoperiodic lighting regime, temperature, the action of growth stimulants and inhibitors, the cationic composition of the environment, and others. A few minutes of the body's stay in unfavorable conditions is enough to restructure the genetic apparatus to ensure the possibility of synthesizing stress proteins (Kosakovskaya, 2008). Molecular analysis of global genome expression revealed that Arabidopsis root cells respond to salinity by altering the transcription of 5590 genes. Moreover, the most serious events in changes in gene expression are observed during the first 3 hours of salt exposure (Geng Yu., 2013). Thousands of NaCl-regulated genes encode ion transporters, ion and water channel proteins, and ATP transport enzymes, which are necessary for restoring the water potential gradient in the soil-plant system disrupted by salinity, maintaining intracellular water homeostasis, and overcoming salt-induced ion imbalance, as well as the biosynthesis of osmoregulators and multifunctional stress-protective compounds (Almikhlafi, 2014). Islam and his colleagues (Islam, 2009) having studied

the effect of abiotic stress on the expression in rice of the gene encoding a protein containing a zinc finger domain, encoding a protein 171 amino acid residues long, noted a strong induction of the expression of the OsZFP gene in the roots and leaves of plants under salinity, and concluded that OsZFP, as a transcription factor, can play an important role in the response of rice to salinity. Analysis of the transcriptome composition of plants exposed to high temperature, drought, cold, salinity, strong light or mechanical stress showed that only a few genes respond to these stresses in a similar way (Kreps et al., 2002) Almost all plants respond to the action of any stressor by activating different groups of genes and synthesizing the protective proteins they encode. Plants contain a huge number of genes regulated by water deficiency and encoding various proteins: regulatory, protective (chaperones), enzymes. In addition to the synthesis of shock proteins, which shows that a special program associated with experiencing stress is recorded in the genome, the content of carbohydrates and proline in cells increases, which participate in protective reactions, stabilizing the cytoplasm. The accumulation of proline as an osmotically active organic substance promotes the retention of water in the cell. The effect of salinity leads to a decrease in free water in cells, which changes the hydration membranes of cytoplasmic proteins and affects the functioning of enzyme proteins (Hayat et all., 2012; Hualpa-Ramirez et al., 2024). The activity of synthesis enzymes decreases and hydrolytic processes are activated, which leads to an increase in the number of low-molecular proteins. As a result of the hydrolysis of polysaccharides, soluble carbohydrates accumulate in the cells, the outflow of which from the leaves is slowed down. The amount of RNA decreases (due to inhibition of its synthesis and activation of ribonucleases). The disintegration of polyribosome complexes is underway. Long-term exposure to stress can cause changes in the structure of DNA. The survival of a plant under conditions of excessive salinity depends not on the tolerance of the proteins themselves, but on their microenvironment, the ability of cells to maintain ionic homeostasis. Compatible osmolytes (sugars, sugar alcohols, free amino acids, etc.) lower the water potential, protect membranes, enzymes, structural and regulatory macromolecules. The increase in the concentration of these substances occurs due to the activation of genes that control enzymes synthesizing osmolytes and the inhibition of the expression of other genes responsible for their destruction (Gold et al., 2008). Salt stress is accompanied not only by osmotic, but also by the toxic effect of excess ion content (Sairam and Tyagi, 2004). High concentrations of salts, a decrease in the osmotic potential of soil solutions, lead to water deficiency in plants, while Na+ and Cl- ions have a direct toxic effect on plant cells. As a result of the interaction of salts with nutrients, an imbalance and nutritional deficiency are observed (Zhu, 2001). It should be noted that the selection of salt-tolerant forms in field conditions is complicated by the uneven distribution of saline areas (Munns, 2005). The study of nucleic acid metabolism is of great importance in connection with the clarification of the mechanisms of adaptation of plant organisms to stress. The aim of this study was to study the adaptive capacity of cotton collection material to salt stress, as well as to study changes in the structural state of DNA and the activity of nucleic acid synthesis in connection with the degree of resistance to salinity stress in cotton collection samples.

Material and Methods

The objects of the study were collection samples of cotton of the species G.barbadense L.

The method of germinating cotton seeds in a solution with an osmotic pressure of 0.2 M NaCl was used as an indicator of plant resistance to salinity (Udovenko, 1988). The percentage of germinated seeds (P) is determined as follows: the average number of germinated seeds in the control is taken as 100%, the average number of germinated seeds in the experiment (a) is expressed as a percentage of the number of germinated seeds in the control (b). Thus, P=a/b 100% The nucleic acid content was determined by DNA fractionation based on the principle of stepwise action on chromatin by solutions of different ionic strength and deproteinization factors, which allows dividing cellular DNA into labile (functionally active) or weakly bound in the chromatin structure and stable, completely blocked by histones, as well as residual or tightly bound. Extraction of labile DNA. Leaves were ground in a mortar with quartz sand in the cold in 5 volumes of a mixture of 0.15 M NaCl; 0.03 M sodium citrate; 0,05M Tris – HCl 8.0; 0.001 M EDTA. The resulting homogenate was mixed in the cold and centrifuged at 500 g for 15 min. The aqueous phase (extract 1) was decanted, and the sediment was re-extracted under these conditions. The combined aqueous-salt layer was again centrifuged at 10,000 g for 15 min. Sediment 2, containing chloroplasts, mitochondria, cell debris and other cytoplasmic particles, was discarded, and the supernatant was precipitated with two volumes of cold ethanol. Extraction of stable DNA. The residue after extraction of labile DNA (precipitate 1) was again homogenized in 6 volumes of a mixture of 0.6 M NaCl; 0.001 M EDTA; 0.05 M Tris-HCI pH 8.0; 2% PAS (sodium para-aminosalicylate); 0.5% SDS (sodium dodecyl sulfate). After 15 min of mixing and subsequent centrifugation at 2500 g, the upper (water-salt) layer was aspirated. The precipitate was washed again with the extractant and the combined aqueous phases were precipitated with two volumes of cold ethanol. Extraction of residual DNA. 0.6 M NaCl extracts the bulk of the cell DNA. However, some of the DNA, especially tightly bound in the cell structures, remains unrecovered. For complete extraction, the residue of plant tissue after stable DNA extraction was suspended in 6 volumes of a mixture of 0.18 MNaCl; 0.001 M EDTA; 0.02 M phosphate buffer pH 8.0; 5% PAS; 1% SDS. The temperature of the mixture was brought to 60 °C and maintained for 5 min, then cooled to room temperature. The centrifugate was precipitated with two volumes of cold ethanol.

Subsequent washing of the alcohol precipitate of DNA fractions was carried out in the following sequence:

1. 80% ethanol 3 times at 80 °C;
2. 5% TCA 2 times in the cold;
3. 80% ethanol at room temperature;
4. 96% ethanol at room temperature;
5. a mixture of ethanol: ether (3:1) at room temperature 65-70 °C 2 times.

The precipitate obtained after such treatment was dissolved in a minimum volume of 0.5 N NaOH and incubated for 18 hours at 37 °C. After the specified time, the hydrolysates were cooled and neutralized with 57% perchloric acid and acidified with it to pH 1.0. They were kept for 30 min (until the DNA precipitate was formed) and centrifuged at 12,000 g for 20-30 min. The centrifugate (RNA) was decanted, and the precipitate was washed again with 0.5 N HClO4. The centrifugates were combined and the amount of RNA was determined after hydrolysis. The precipitate was poured with 3-5 ml of 0.5 N HClO4, hydrolyzed for 20 min in a boiling water bath and, after cooling and making the appropriate dilution, spectrophotometered at wavelengths of 270 and 290 nm.

Results

A certain role in identifying the traits that determine a form or variety in relation to its resistance to unfavorable environmental factors belongs to the physiological characteristics of the plant. The physiological response to stress is an emergency mobilization of the adaptive potential, ensuring temporary survival of adverse effects and, in this regard, having adaptive

significance.. Since differences in the mechanisms of perception and transduction of stress signals in plants lead to different tolerance to stress, the study of the stress response allows us to identify the comparative degree of plant resistance to the action of abiotic environmental factors. Cotton is particularly sensitive to the effects of unfavorable environmental factors, and therefore has the least resistance, at the germination stage (Akparov et al., 2006). In this regard, we studied the adaptive potential of collection varieties of cotton in terms of seed germination under salt stress. The study of the degree of resistance of cotton samples to salinity showed that for different cotton varieties, due to genetic specificity, the influence of the stress factor is not the same. Depending on the genotype, variety samples of the same species differed significantly in the amplitude of the physiological parameter during adaptive processes. The reaction of variety samples to the action of unfavorable environmental factors allowed us to roughly divide variety samples into groups within the species, determining different degrees of comparative resistance.

Cluster Analysis of Resistance to Salinity of Cotton Variety Samples Is Presented in The Figure 1

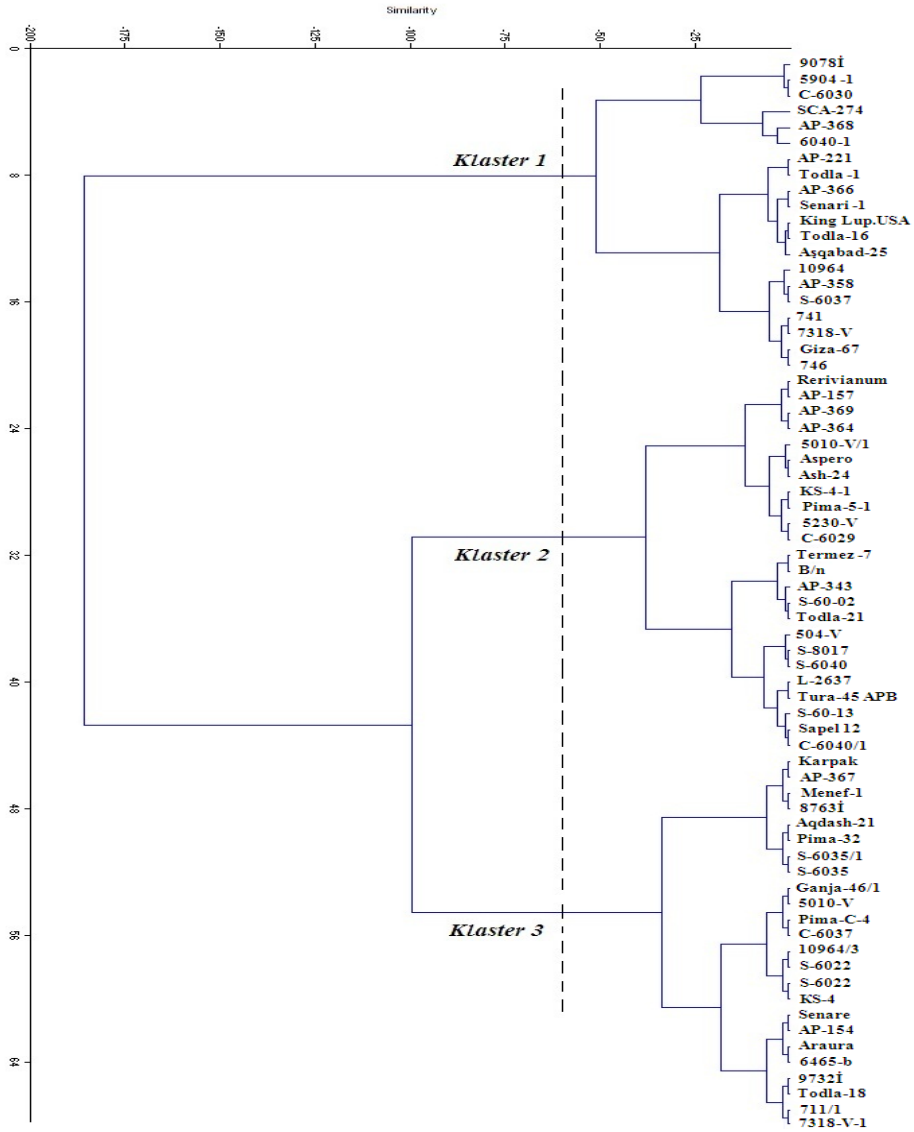

Figure 1: Distribution of cotton varieties of the species G. barbadense L. by degree of resistance to salinity.

Highly resistant to stress cotton varieties were grouped in cluster 3. High resistance of cotton varieties to stress determines their ability to maintain a normal metabolic rate with a wider range of values of the unfavorable factor and a higher rate of development of protective metabolic changes. Resistant plants, compared to unstable ones, most fully rebuild their vital functions in the direction of adaptation to unfavorable environmental conditions. Unresistant plants under the influence of a negative environmental factor are more conservative and are not capable of quickly changing their vital functions, as a result of which they often die. Thus, as a result of the research it was established that with the same intensity of the extreme factor, the varieties of the same type of cotton differ significantly in the amplitude of the change in the physiological parameter, which made it possible to identify highly resistant and sensitive genotypes. The lever for implementing adaptive restructuring is the metabolic coordination system, ultimately controlled by the center of gene regulation of nuclear DNA, realized through the activity of enzymatic systems and limited by the energy potential of the cell and the organism as a whole. There is information in the literature that abiotic stressors express a multigene system (Kotak et al., 2007). Since each physiological mechanism is determined by its own gene complexes or blocks, it seemed important to us to study the nature of changes in the

synthesis of nucleic acids and the fractional composition of DNA under stressful conditions in cotton varieties characterized by varying degrees of resistance to abiotic stress factors. As the results of the study showed, cotton varieties differed in their resistance to salt stress. The varieties AP-154, 9732I, 5010-V, Senare, S-6022 and S-6002 are highly resistant to salinity. Stress depression of seed germination in a salt solution is completely absent in these samples (Table 1). Under stress conditions, salt-tolerant varieties show activation of RNA synthesis, although to varying degrees, depending on the genotype of the plants. The increase in RNA content in the studied experimental varieties compared to the control plants ranged from 5.6 to 31.1%. For example, in the 9732I variety, the increase in RNA synthesis under salinity was 7.0%, and in the Senare variety, 20.9%. The structural state and functional activity of the DNA of the cell nucleus is heterogeneous (Konarev, 1970). Part of it is in a labile state as part of dispersed chromatin – euchromatin and is functionally more active. The DNA of labial chromatin is unsaturated with histones, has many free phosphate groups and metastable, easily denatured regions. The majority of DNA is tightly bound to histones and is a component of less active compact chromatin.. Stable chromatin belongs to metaphase chromosomes and compact structures of interphase chromosomes, including heterochromatin.. It is poor in non-histone proteins and RNA. DNA in stable chromatin is saturated with histones and relatively poor in free phosphate groups. A small part of DNA (perinucleolar chromatin) is firmly bound in chromatin due to special packaging in structures containing RNA and lipids in addition to proteins. Residual chromatin contains non-histone proteins that are common with labile chromatin but absent from stable chromatin. A study of the nature of nucleic acid synthesis and the fractional composition of DNA under salt stress showed that, in addition to RNA, stress-resistant varieties showed an increase in total and labile DNA. For example, in control plants of the Senare variety, the total DNA content was 16.102 mg%, labile DNA – 5.107 mg%, in experimental plants of this variety, the activation of the synthesis of these indicators was 6.611% and 6.215%, respectively. At the same time, salt-tolerant varieties show a decrease in the amount of stable DNA when exposed to salinity. For example, under stress, the percentage of stable DNA in the Senare variety decreases by 7.1%. The same picture is observed in other stress-resistant variety samples (figure 2). As for residual DNA, salt-tolerant samples showed an increase in its quantity. The only exception was the 9732I variety, in the experimental plant of which the decrease in residual DNA was 0.9%.

№	Varieties	Seed germination under salinity, in%				RNA, mg%		DNA, mg%		
		control	experience	in % of control	stress-depression, in %	control	experience	DNA fractions	control	experience
1.	AP-154	76,0	76,0	100	0	105,0±4,35	137,63±1,29	labile	4,274±0,11	5,657±0,04
								stable	7,209±0,04	6,310±0,09
								residual	1,224±0,06	1,472±0,06
								total	12,707	13,439
2	9732I	92,0	92,0	100	0	117,944±2,42	126,224±2,87	labile	5,506±0,11	5,941±0,08
								stable	7,794±0,14	7,244±0,14
								residual	2,252±0,04	2,226
								total	15,552	15,411
3.	5010-V	100	100	100	0	107,64±5,18	113,71±2,72	labile	5,595±0,07	6,207±0,09
								stable	7,661±0,22	7,067±0,08
								residual	2,299±0,07	2,509±0,06
								total	15,555	15,783
4.	Senare	94,8	94,8	100	0	122,36±2,434	147,93±1,35	labile	5,107±0,08	6,215±0,15
								stable	8,494±0,10	7,891±0,10
								residual	2,501±0,03	2,505±
								total	16,102	16,611
5.	S-6022	95,0	95,0	100	0	123,832±1,50	147,016±2,48	labile	5,187±0,08	6,242±0,05
								stable	9,283±0,11	8,503±0,08
								residual	2,021±0,04	2,385±0,05
								total	16,491	17,130

Table 1: Changes in seed germination, RNA content and DNA fractions in stress-resistant cotton varieties under salinity conditions.

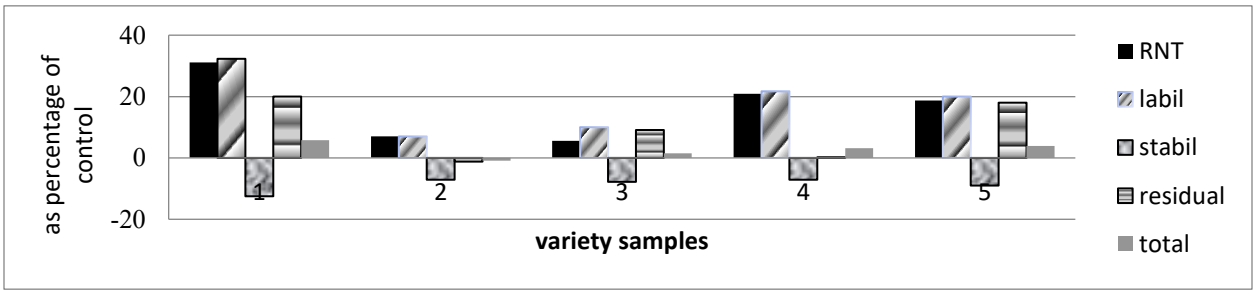

Figure 2: Changes in nucleic acid synthesis under salinity in stress-resistant varieties.

AP-154 2. 97321 3.5010-V 4. Senare 5.S-6022

In cotton genotypes characterized by stress depression of seed germination in a saline solution, a decrease in the synthesis of RNA, total and labile DNA is observed in comparison with control plants (Table.2). Moreover, the more sensitive the plant was to salinity, the greater the decrease in RNA, total and labile DNA synthesis was. For example, stress depression of seed germination under salinity in variety 741 was 15%, depression of labile DNA synthesis in experimental plants, compared to the control, was 9.5%. In variety C-6040-1, depression of seed germination under stress was 49.2%, depression of labile DNA synthesis was 18.6%. At the same time, this process was accompanied by an increase in the amount of stable DNA (figure 3).

Discussion

In adaptive metabolic restructuring, a major role is played by a more complete mobilization of the potential capabilities of various reactions, compensatory changes in associated processes, and the inclusion of shunt mechanisms (Udovenko, 1995). Under normal conditions, the intensity of various processes occurs at levels significantly lower than the maximum possible. Under unfavorable conditions, these capabilities are used more fully, which allows plants to maintain the speed of many reactions at a fairly high level. Differences between varieties in terms of resistance level are genetically determined and are hereditarily preserved over a number of generations. Since differences in the mechanisms of perception and transduction of the stress signal in plants lead to different tolerance to stress, the study of the stress response allows us to identify the comparative degree of plant resistance to the action of abiotic environmental factors. As a result of the studies, it was found that with the same intensity of the extreme factor, variety samples of the same cotton species differ significantly in the amplitude of the change in the physiological parameter. The amplitude of physiological parameters under stress depends on the level of plant resistance, which is an inherited potential ability of the organism to adapt and is realized under the action of an extreme factor. The reaction of the variety samples to the action of unfavourable environmental factors made it possible to divide the variety samples into groups within the species, determining different degrees of comparative resistance. In an unfavorable situation, the rapid response of plants is gene expression. A study of the expression of potential defense reaction genes in cotton showed that its regulation is more pronounced and occurs more quickly in the resistant variety (Cui, 2000). In our studies, the effect of stress on salinity-resistant cotton varieties causes activation of the synthesis of total, labile and residual DNA, as well as RNA, which indicates an increase in the physiological lability and functional activity of the genetic apparatus. Chromatin labilization is promoted by factors that stimulate growth and metabolic processes in the body. Despite the fact that the content of residual DNA in the total amount of the genome is the smallest, there is an assumption that it is metabolically active and plays a role in accelerating cell division (Konarev, 1970).

№	Сорта	Seed germination under salinity, in%				RNA, mg%		DNA, mg%		
		control	experience	in % of control	stress-depression, in %	control	experience	DNA fractions	control	experience
1.	S-6002	92,0	86,8	94,3	5,7	109,48±2,55	102,304± 1,75	labile	4,699±0,02	4,501±0,11
								stable	7,253±0,07	7,341±0,08
								residual	1,489±0,05	1,472±0,06
								total	13,417	13,314
2	741	80,0	68,0	85,0	15,0	112,056±1,59	102,672±1,69	labile	4,859±0,08	4,398±0,11
								stable	7,093±0,09	7,404±0,09
								residual	2,101±0,04	2,004±0,05
								total	14,053	13,806
3.	5904-1	78,5	58,5	74,5	25,5	120,152±1,44	104,328±2,40	labile	5,533±0,03	4,833±0,08
								stable	7,652±0,08	8,007±0,06
								residual	2,341±0,03	1,986±0,02
								total	15,526	14,826
4.	C-6040-1	73,2	37,2	50,8	49,2	125,304±2,60	101,752±1,22	labile	5,905±0,11	4,806±0,05
								stable	8,406±0,08	7,767±0,06
								residual	2,500±0,05	2,013±0,07
								total	16,811	14,586

Table 2: Changes in seed germination, RNA content and DNA fractions in stress-sensitive cotton varieties under salinity conditions.

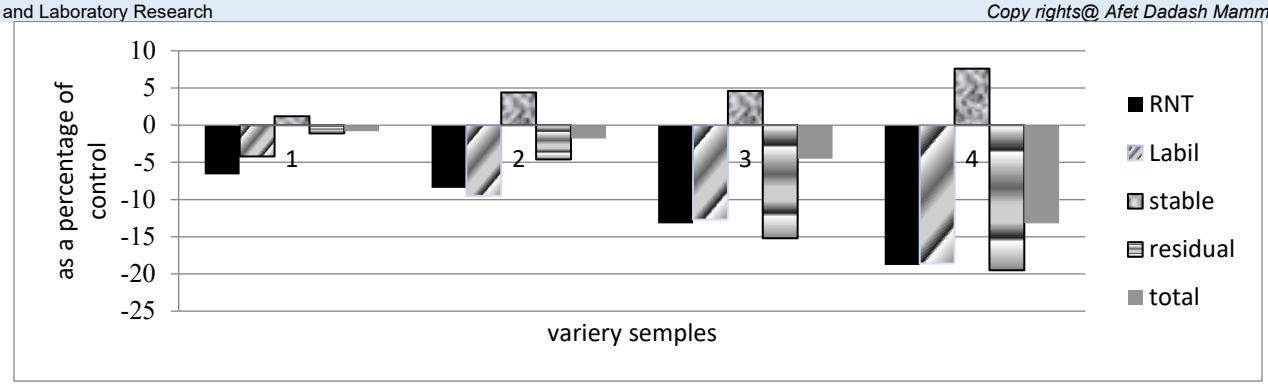

Figure 3: Changes in nucleic acid synthesis under salinization in cotton varieties characterized by different sensitivity to stress

1.C-6002 2. 741 3.5904-1 4.C-6040-1

The increase in the content of labile DNA and RNA in salt-tolerant cotton samples under stress indicates that stress-resistant genotypes exhibit an increase in the physiological lability of the genetic system. A positive change in the synthesis of labile, residual DNA and RNA in stress-resistant samples indicates an increase in the functional activity of the genome, which in turn ensures the acceleration of synthetic processes, especially protein synthesis, and thereby increases the body's resistance to stress factors. In stress-unstable samples, a decrease in RNA and DNA synthesis and a shift in the ratio of DNA fractions towards the stable one are observed. It is known that DNA stabilization is promoted by factors that inhibit growth and metabolic processes. Stable chromatin is not characteristic of the nuclei of embryonic cells capable of reproduction by mitosis. It usually accumulates most in the nuclei of cells in a state of rest, in seeds, dormant buds, etc. Depression in the development of stress-sensitive varieties is caused by changes in the structural state of total DNA and the functional activity of nuclear DNA - the transition from a loose (labile DNA) to a less active state tightly packed with histones (stable DNA). Blocking DNA with histones ensures its increase to unfavorable factors of influence. At the same time, in such a stabilized structural state, the functional activity of DNA decreases, in particular, its activity as a general regulator of synthetic reactions of metabolism. The obtained data on the change in the ratio of DNA fractions are consistent with the literature data on the study of the effect of cold stress on the change in the ratio of eu- and heterochromatin in the nuclei of soybean plant root cells (Stepinski, 2012). The author found that under cold conditions the fluorescence intensity for markers characteristic of heterochromatin increased, while for markers corresponding to euchromatin it decreased. After restoration of optimal conditions for plant growth, the opposite situation was observed, that is, an increase in the fluorescence intensity of markers corresponding to the euchromatic fraction and a decrease in the fluorescence intensity of markers corresponding to the heterochromatic fraction were noted. Thus, the data we have obtained indicate that stress factors affect the structure and functional activity of the plant genome. If this effect does not have a negative effect on stress-resistant varieties, then they have a negative effect on sensitive genotypes and cause degradation of nucleic acids, which leads to weakening of plants, decreased resistance and can even lead to the death of the plant.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Ramiz Aliyev played a pivotal role in formulating the original research question and offering invaluable direction and guidance throughout the investigation. Afet Dadash Mammadova and Ramiz Aliyev conducted thorough data analysis and collaborated closely to write the manuscript. All authors read and approved the final manuscript.

Data Availability

All data generated during this research are included in this published article and its supplementary information files.

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