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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

MOLECULAR GENETIC STUDY OF THE RESISTANCE OF COTTON GENOTYPES TO SALINITY STRESS

Speciality: 2409.01 – “Genetics”

Field of Science: Biology

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Baku – 2026

The work was performed at the departments of "Industrial and forage crops", "Physiological genetics", and "Molecular genetics and genomics" of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

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INTRODUCTION

Relevance and degree of development of the topic. Agriculture is one of the main profitable sectors of the economy of developing countries, and cotton is a particularly important crop in these countries. Cotton, which has a natural and high fiber quality, is the main raw material of the textile industry, which has a high economic impact[1].

Approximately 20% of cultivated and 33% of irrigated land worldwide are affected by salinity[2]. Soil salinization is spreading globally at an average rate of 1-2 million hectares per year, affecting a significant portion of crop production and rendering land unsuitable for cultivation[3]. Across the republic, areas exposed to salinity account for 12.5% of total agricultural lands. As a result of climate change, increased use of poor-quality water for irrigation, and intensive farming, more than 50% of agricultural land is likely to become salinized by 2050, which will result a serious threat to the sustainability of agriculture[4,5]. Developing salt-tolerant crops will contribute to the sustainable development of agriculture in salt-affected areas, the reclamation of these lands, and at the same time, the improvement of the livelihoods of the population living in those areas[6].

Investigating the response of cotton plants to salinity, its resistance mechanisms, and management methods can help formulate strategies to

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[1]Ahmad, S. Cotton Production and Uses / S. Ahmad, M. Hasanuzzaman – Singapore: Springer Nature – 2020. – 650 p.

[2] Karaca, C. Effects of Salinity Stress on Drip-Irrigated Tomatoes Grown under Mediterranean-Type Greenhouse Conditions / C.Karaca, G.E.Aslan, D.Buyuktas [et al.] // Agronomy, – 2023. 13(1), – p. 1-13.

[3] Devkota, K.P. Managing salinity for sustainable agricultural production in salt-affected soils of irrigated drylands / K.P.Devkota, M.Devkota, M.Rezaei [et al.] // Agricultural Systems, – 2022. 198, – p. 1-17.

[4] Zeng J. Disomic Substitution of 3D Chromosome with Its Homoeologue 3E in Tetraploid *Thinopyrum elongatum* Enhances Wheat Seedlings Tolerance to Salt Stress / J.Zeng, C.Zhou, Z.He [et al.] // *International Journal of Molecular Sciences*, – 2023. 24(2), – p. 1-13.

[5] Butcher, K. Soil Salinity: A Threat to Global Food Security / K. Butcher, F.W. Abbey, D.Thomas [et al.] // Agronomy Journal, – 2016. 108(6), – p. 2189-2200.

[6]Rahman, M. Cotton Precision Breeding / M.Rahman, Y.Zafar, T.Zhang, - Switzerland: Springer Cham – 2021. – 460 p.

improve cotton productivity under salinity conditions[7]. Sustainable selection for similar traits has led to a narrowing of the genetic base in cotton, as well as the diversity of some traits, such as salt and drought tolerance and disease resistance[8]. Cultivated cotton has a narrow genetic base, and therefore, expanding the genetic base of cultivated cotton by transferring beneficial genetic variations from various exotic and wild species is one of the top priorities[9].

The completion of the whole genome sequencing of various cotton species offers new model systems for a better understanding of adaptive mechanisms in extreme environments. Transcriptome analysis is widely used to screen candidate genes involved in stress responses by providing detailed information on gene expression at the mRNA level, and to investigate the molecular regulatory pathways of salt stress tolerance and adaptation in cotton[10]. Currently, only a small number of genes that respond to high salt concentrations have been studied in cotton [11].

For the rational use of cotton collection samples stored in the National Genebank in genetic-selection programs, the evaluation of genotypes through genetic statistical models are highly relevant, allowing to increase the intensity and effectiveness of selection for various traits. In this regard, the presented dissertation work is dedicated to solving a pressing issue – a comprehensive study of the activity of stress-associated genes and salt tolerance in cotton genotypes under normal and salinity stress conditions.

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[7]Sharif, I. Salinity stress in cotton: effects, mechanism of tolerance and its management strategies / I.Sharif, S.Aleem, J.Farooq [et al.] // Physiol Mol Biol Plants, – 2019. 25(4), – p. 807-820.

[8] Maryum, Z. An overview of salinity stress, mechanism of salinity tolerance and strategies for its management in cotton / Z.Maryum, T.Luqman, S.Nadeem [et al.] // Front. Plant Sci., – 2022. 13, – p. 1-22.

[9] Yadava, D.K., Dikshit, H.K., Mishra, G.P., Tripathi, S. Fundamentals of Field Crop Breeding / D.K. Yadava, H.K. Dikshit, G.P. Mishra, S. Tripathi. – Singapore: Springer Nature, – 2022. – 1389 p.

[10]Wei, Y. Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis / Y.Wei, Y.Xu, P.Lu [et al.] // PLoS ONE, – 2017. 12(5), – p. 1-25.

[11] Taghizadeh, N. Salt-related Genes Expression Pattern in Salt-Tolerant and Salt-Sensitive Cultivars of Cotton (*Gossypium* sp.) under NaCl Stress / N.Taghizadeh, G.Ranjbar, G.Nematzadeh [et al.] // J. Plant Mol. Breed., – 2018. 6(1), – p. 1-15.

Object and subject of the research. The object of the study was 13 local and 35 introduced varieties and lines of cotton plants, and the subject was morphophysiological and molecular genetic study of salt resistance in cotton.

Purpose and tasks of the research. The purpose of the research was to study the transcripts of stress-associated genes in geographically distant cotton genotypes, study the effect of different concentrations of NaCl salt on the biometric indicators of cotton genotypes, compare genotypes based on stress resistance indices, and study the dynamics of changes in the expression levels of stress-associated genes in resistant and sensitive genotypes. To achieve this goal, the following tasks were set:

1. Comparative analysis of transcripts of genes encoding MAPK, CIPK, antiporter, reactive oxygen species (ROS) scavenging genes and members of the gene family encoding of various transcription factors under normal and salt stress conditions;

2. Comparative study of the expression of stress-associated genes between the chromosome substitution lines and parental forms;

3. Determination of the specificity of the response of genotypes to NaCl salt by comparative analysis of biometric parameters *in vitro*, comparative study of genotypes based on tolerance indices;

4. Comparative study of the expression of stress-associated genes in groups differing in salt tolerance;

Research methods. Classical (analysis of morphophysiological characteristics) and modern methods (RNA extraction, cDNA synthesis, RT-PCR, etc.) were used in the research work.

The main provisions of the defense:

1. There is statistically significant difference among 48 geographically distant cotton genotypes regarding their morpho-physiological and genetic parameters under control conditions and under stress induced by 100 mM and 200 mM NaCl concentrations.

2. Based on the dynamics of their expression patterns, the chromosome substitution lines exhibit both similarities and differences among themselves and in comparison to their parental forms.

3. The genes *GhSOD* and *GhWRKY39* in line 4, *GhMT3a*, *GhNHX1* and *GhMAPK* in line 12, *GhFSD1* in line 14, *GhCIPK6* in line 15, *GhDi19-1* in line 17, *GhCAT1* and *GhERF2* in line 22, and *GhAPX1*,

GhDREB and *GhDi19-2* in line 25 developed via chromosome introgression exhibit higher expression levels compared to other lines.

4. Among the genotypes selected based on biometric indicators – whether tolerant or sensitive – there are distinct expression dynamics for genes involved in the regulation of reactive oxygen species (with the exception of *GhMT3a* in sensitive genotypes), as well as for genes encoding antiporters, MAPK, CIPK, and the investigated transcription factors.

5. The STI, MP, YI, and GMP indices are effective indicators (with statistical reliability) for identifying cotton genotypes tolerant to NaCl salt.

6. Under stress conditions induced by 100 mM and 200 mM NaCl concentrations, the total fresh and dry height and weight of cotton plants can serve as distinguishing indicators for identification.

The scientific novelty of the research. For the first time, the morphophysiological and genetic characteristics of 48 local and introduced varieties and lines of cotton belonging to *G. barbedense* L. and *G. hirsutum* L. were studied in a comprehensive manner under normal, 100 mM and 200 mM NaCl concentrations conditions, and it was determined that their biometric parameters changed. Salt tolerance was assessed, and the expression level of 13 genes was studied comparatively. According to the least significant difference test (LSD), the expression level of genes associated with stress in the varieties, chromosome substitution lines, and their parents was found to be statistically significant ($P \leq 0.05$). For the first time, CSLs exhibiting higher expression levels of the *GhSOD*, *GhCAT1*, *GhMT3a*, *GhAPX1*, *GhFSD1*, *GhDREB*, *GhDi19-1*, *GhDi19-2*, *GhERF2*, *GhWRKY39*, *GhMAPK*, *GhCIPK6*, and *GhNHX1* genes compared to the donor line (*G. barbadense* L.) have been identified.

For the first time, the correlation between the increase in salt concentration and the change in germination parameters was studied in cotton. To determine the presence of a significant difference between the average indicators of the groups, a t-test was performed, and a significant difference in the change in biometric parameters was found.

For the first time, a statistical analysis of the relationships between productivity and tolerance indices under stress conditions was performed for the chromosome-replaced lines and their parents, with the control group, and it was found that STI, MP, YI, and GMP indices were effective resistance indices for the identification of salt-tolerant genotypes under

stress conditions of 100 mM and 200 mM NaCl salt concentrations.

The scientific and practical significance of research. The results obtained in the research work create conditions for a deep understanding of the morphophysiological indicators and molecular genetic mechanisms of salt stress resistance in cotton plants at different concentrations of NaCl, and are also important in the identification of new varieties and forms resistant to biotic and abiotic stress factors, in the comprehensive characterization of cotton collection samples, in the intensification and increase of the efficiency of the selection process, serving the preservation and enrichment of genetic diversity. For the first time, a comprehensive assessment of the salt resistance of 48 local and introduced cotton genotypes belonging to the National Genebank operating at the Institute of Genetic Resources of the Ministry of Science and Education at the morphophysiological and molecular-genetic levels will create conditions for their effective use in the creation of salt-resistant varieties in genetic, ecological, and selection programs, and in the identification of new hybridization combinations of genetically distant forms.

Approbation of the work. 18 scientific works, including 10 articles (8 of which are in periodical scientific publications included in the international abstracting and indexing database), and 8 theses, have been published in local and foreign publications on the results of the dissertation work. The main results of the presented dissertation work were presented at the Republican Scientific-Practical Conference of Doctoral Students and Young Researchers Dedicated to the 99th Anniversary of the National Leader Heydar Aliyev in Improving the Environment in Azerbaijan (Baku, 2022), the VI International Scientific Conference of Young Researchers Dedicated to the 99th Anniversary of the National Leader Heydar Aliyev (Baku, 2022), the International Conference "Heydar Aliyev and the Development of Modern Biological Science: Achievements and Challenges" (Baku, 2023), the International Scientific Conference on Current Problems of Modern Natural and Economic Sciences Dedicated to the 101st Anniversary of the National Leader Heydar Aliyev (Ganja, 2024), the Scientific-Practical Conference on "The Role of the National Leader Heydar Aliyev in Improving the Environment in Azerbaijan" (Baku, 2024), at the VIII International Scientific Conference of Young Researchers dedicated to the 101st anniversary of the National Leader Heydar

Aliyev (Baku, 2024), LXIII International Multidisciplinary Conference "Recent Scientific Investigation" (Shawnee, USA, 2024) and at scientific seminar of Baku State University.

Organization in which the dissertation was performed. The dissertation work was carried out in the departments of "Industrial and forage crops", "Physiological genetics", "Molecular genetics and genomics" of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

Total volume of the dissertation with the number of characters indicating the volume of the structural sections of the dissertation. The dissertation consists of 205 pages, including an introduction, 5 chapters, conclusion, results, recommendations, bibliography, appendicies and a list of abbreviations and symbols. The total volume of the dissertation is 193 356 characters (Introduction – 9 868, chapter I – 47 555, chapter II – 16 473, chapter III – 45 694, chapter IV – 49 006, chapter V – 15 115, conclusion – 7 200, results – 2 010, recommendations – 435). The work includes 27 tables and 31 figures presenting the findings, and references 249 sources.

MAIN CONTENT OF THE DISSERTATION
CHAPTER I. LITERATURE REVİEW

In the literature review of the dissertation, cotton cultivation and its economic importance, salt tolerance of various cotton species, the effect of salt stress on various morphophysiological and genetic parameters in cotton, salt stress management strategies, transcriptome analysis of salt tolerance in cotton and the importance of various genes associated with stress in salt tolerance were extensively analyzed, and references to local and foreign literature sources were provided.

CHAPTER II. MATERIALS AND METHODS OF RESEARCH

32 genotypes of the cotton belonging to species *G. hirsutum* L., 1 genotype belonging to species *G. barbadense* L., and 15 chromosome substitution lines were used as a research material.

Testing of salt tolerance in laboratory conditions based on germination parameters was performed according to the Kader[12] method. The salt tolerance of the genotypes was studied in terms of biomass production and reduction rates of the samples at 100 mM and 200 mM NaCl concentrations in a completely randomized design[13]. Stress tolerance indices were calculated based on the dry mass of cotton seedlings according to the methodology of Fischer and Fernandez. SPAD values were recorded according to the methodology proposed by Rosyara using a Minolta chlorophyll meter to measure chlorophyll content without disturbing leaf under control and stress conditions [14].

For transcriptome analysis, total RNA was extracted from leaf of seedlings samples collected in control and stress variants using the RNX-Plus Solution (Cat. No. EX6101) protocol. SinaClon First Strand cDNA (Cat. No. RT5201) synthesis kit was used to synthesize full-length mRNA cDNA. The quality of RNA and cDNA was checked using NanoDrop 2000/2000c. To carry out primer design, complete coding sequences were selected from the NCBI and were carried out using the online web resources Primer3web and Primer3Plus. The base and salt-adjusted temperatures of the primers were checked using the online Oligo Calculator web resource. To check the uniqueness of the primers, Blast nucleotide was performed using the online web resource. RT-PCR was optimized using

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[12] Kader, M.A. A Comparison of Seed Germination Calculation Formulae and the Associated Interpretation of Resulting Data // Journal & Proceedings of the Royal Society of New South Wales, – 2005. 138, – p. 65-75.

[13] Shaheen, S. Effect of NaCl on Morphophysiological and Biochemical Responses in *Gossypium hirsutum* L. / S.Shaheen, M.Baber, S.Aslam [et al.] // Agronomy, – 2023. 13(4), – p. 1-19.

[14] Rosyara, U.R. Photochemical Efficiency and SPAD Value as Indirect Selection Criteria for Combined Selection of Spot Blotch and Terminal Heat Stress in Wheat / U.R.Rosyara, S.Subedi, E.Duveiller [et al.] // Journal of Phytopathology, – 2010. 158(11-12), – p. 813-821.

gene-specific primers, and a 2x SYBR Blue HS-qPCR kit (Cat. No. MM2171) was used during the preparation of the mix. The formula proposed by Pfaffl was used to check the amplification efficiency of the 2 amplicons [15]. Microsoft Excel v2010 was used to calculate the results of genotype resistance assessment and gene expression ratio. Pearson correlation coefficient, Kaiser-Meyer-Olkin and Bartlett test for Principal Component Analysis (PCA), and the calculation of the eigenvalues and percentage of variance of components were calculated using IBM SPSS Statistics v.26 software. Biplots were constructed using Past/v.4.17 software. Dendrograms were constructed using the iTOL v6 online web resource.

CHAPTER III. EVALUATION OF THE EXPRESSION LEVEL OF GENES ASSOCIATED WITH SALT STRESS BASED ON TRANSCRIPTOME ANALYSIS

3.1. Evaluation of the expression levels of genes involved in the regulation of ROS under salt stress. The least significant difference (LSD) test, used in the context of variance analysis, is a test procedure for pairwise comparisons of several treatment groups, and helps to identify groups whose means are statistically different. For the first time in the study, based on the results of the LSD test on the control and stress-treated variants, it was determined that there was a significant difference (significant at the 0.05 level) in the expression levels of the *GhSOD*, *GhCAT1*, and *GhMT3a* genes involved in the regulation of ROS between the control and both stress variants, and in the expression levels of the *GhAPX1* and *GhFSD1* genes under the control and 200 mM NaCl stress conditions. At the same time, the highest expression levels of *GhSOD*, *GhAPX1*, *GhFSD1*, *GhCAT1*, *GhMT3a* genes were determined in CS B-11, CS B-26, CS B-15, CS B-15, CS B-02 lines at 100 mM NaCl concentration, and in CS B-04, CS B-25, CS B-14, CS B-22, CS B-12 lines at 200 mM NaCl concentration, respectively, compared to the control.

3.2. Evaluation of the expression level of transcription factors encoding genes under stress conditions. According to the results of the LSD test, there was a significant difference (mean difference significant at

[15] Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT-PCR // Nucleic Acids Research, – 2001. 29(9), – p. 2002-2007.

the 0.05 level) between the expression level of the *GhDi19-1* gene under the control and 100 mM NaCl stress conditions, and a significant difference (mean difference significant at the 0.05 level) between the expression levels of the *GhDREB*, *GhERF2*, and *GhDi19-2* genes under the control and 200 mM NaCl stress conditions. The maximum increase in the expression level of the GhWRKY39, GhERF2, GhDREB, *GhDi19-1*, *GhDi19-2* genes was found in CS B-26, CS B-05, CS B-25, CS B-15, CS B-11 lines at 100 mM NaCl concentration, and in CS B-04, CS B-22, CS B-25, CS B-17, CS B-25 lines at 200 mM NaCl concentration compared to the control.

3.3. Evaluation of the expression level of antiporter (*GhNHX1*), calcineurin B-like interacting protein kinase 6 (*GhCIPK6*) and mitogen-activated protein kinase (*GhMAPK*) encoding genes under salt stress. In the study, under stress conditions with both 100 and 200 mM NaCl salt concentrations, the expression level of *GhNHX1* and *GhMAPK* genes was determined to increase maximally in CS B-12, and the expression level of *GhCIPK6* gene was determined to increase maximally in CS B-15 line compared to the control. Also, according to the results of variance analysis, there was a significant difference in the expression level of *GhNHX1* and *GhCIPK6* genes under control and 200 mM NaCl salt concentration stress conditions.

Analysis of the dynamics of changes in the expression level of the genes studied with increasing stress dose in the study showed that the change in the expression level of the *GhAPX1* gene in the Pima 3-79 genotype does not resemble any line.

CHAPTER IV. EVALUATION OF SALT TOLERANCE OF COTTON GENOTYPES IN LABORATORY CONDITIONS

4.1. Evaluation of durability based on seed germination parameters at different salt concentrations. According to Pearson correlation, as a result of the analysis of the effect of salt concentration on genotypes and germination parameters, a positive correlation was found between salt concentration and mean germinatin time (MGT) at a 99 percent probability level, and a negative correlation between final germination percentage (FGP), germination rate index (GRI), germination index (GI) and

Kotowski's coefficient of velocity (CVG) parameters. In addition, it was found that a decrease of relative final germination percentage (RFGP) of the studied varieties at the concentration of 50 mM of NaCl was between 2-23%, 11-42% at a concentration of 100 mM, 24-56% at a concentration of 150 mM, and 35-71% at a concentration of 200 mM, while in chromosome susbtitution lines (CSLs) and in their parental forms, RFGP was decrased 2-12% under 50 mM, 11-37% at 100 mM, 28-61% at 150 mM and 46-77% at 200 mM concentration of NaCl. Although the MGT was increased compared to the control in all studied genotypes, the degree of increasing MGT was different in various genotypes. A clear negative correlation was observed with increasing salt concentration for CVG except for CS B-02, CS B-17, CS B-18, CS B-26, Prime, CSN-12, Edessa, Lima Karabakh-12 genotypes. Since increasing salt stress led to a decrease in FGP and an increase in germination time, a decrease in GRI was found with increasing salt concentration for all studied varieties and lines.

In terms of germination index, which is a comprehensive measurement parameter that combines germination percentage and rate and is important for accurate assessment in the context of the tested parameters, the local Agdash-3, AP-317, Karabakh-11 and Karabakh-12 genotypes (340, 343, 381, 357, respectively) and the introduced Navai-9, Kyrgyzstan-174, Tashkent-2 and Flash genotypes (403, 360, 353, 341, respectively) showed the highest resistance to salt stress. Among the chromosome substitution lines, CS B-05, CS B-11, CS B-14 and CS B-17 had the highest GI index, with values of 345, 334, 336, 303, respectively, and were found as highly resistant.

4.2. Assessment of salt tolerance of genotypes based on changes in total chlorophyll index. 35 days after stress treatment, a decrease in the total chlorophyll content (TCC) of 200 mM NaCl salt was found in all studied genotypes compared to the control. Only in the local Barakat variety, the TCC was slightly higher at 200 mM salt concentration than at 100 mM salt concentration, but the TCC was lower than the control variant at both salt concentrations.

As a result of cluster analysis of genotypes based on the decrease in the total chlorophyll content, it was determined that the studied varieties were grouped into 3 main groups (Figure 4.2.1).

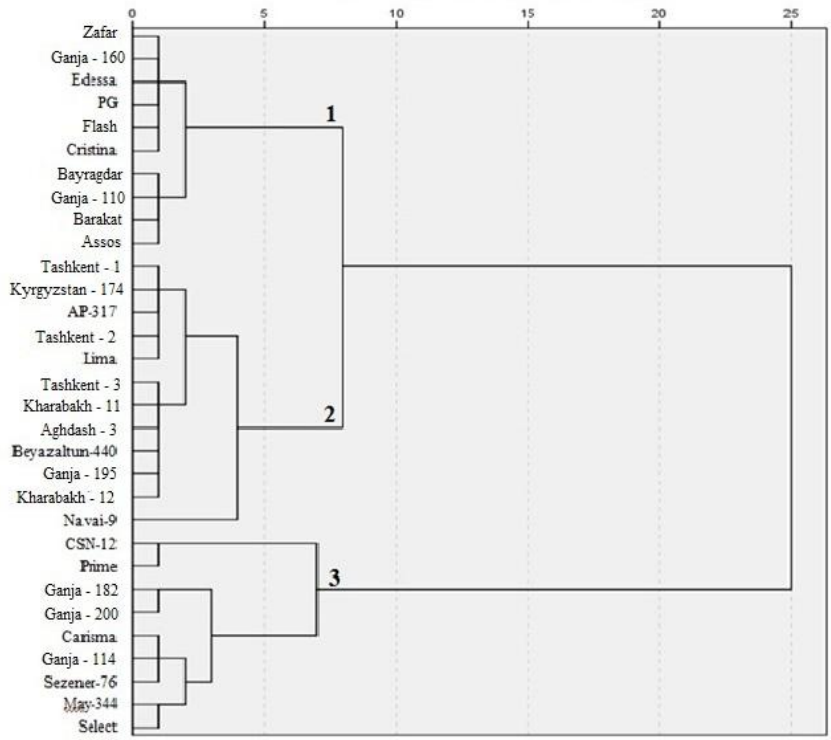


Figure 4.2.1. Grouping of varieties based on changes in chlorophyll content.

In the first group (medium-resistant), the decrease in the total chlorophyll index compared to the control ranged between 4.05-4.675, in the second group (resistant) between 1.6-3.825, and in the third group (sensitive) between 5.225-8.7.

Based on the change in the chlorophyll content of the chromosome substitution lines and parental forms at a concentration of 200 mM NaCl, the studied varieties were grouped into 3 main groups as a result of cluster analysis (Figure 4.2.2).

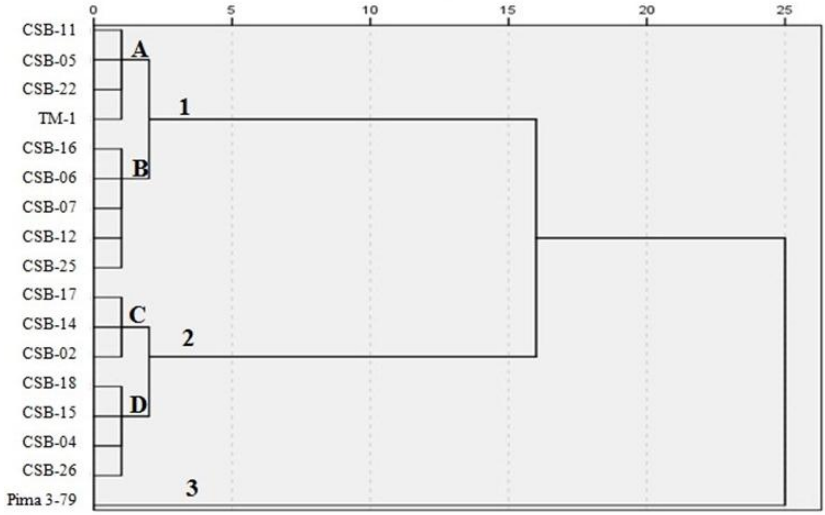


Figure 4.2.2. Grouping of chromosome substitution lines and their parental forms based on changes in chlorophyll content

According to the decrease in TCC, group III was determined as resistant, group I as moderately resistant, and group II as susceptible. At the same time, subgroup 1B (6-6.4) was more resistant than subgroup 1A (6.7-7.1), and 1D (7.4-7.6) was more resistant than 1C (8.2-8.4).

4.3. Assessment of salt stress resistance of genotypes based on morphometric parameters. At 100 mM NaCl concentration, a decrease in the fresh shoot height (FSH) was observed in all varieties and CSLs except for the CSN-12 variety. The decrease in this parameter for the varieties was between 1.4-30.4%, and for CSLs and their parents 2.47-55%. The fresh shoot weight (FSW) increased in the range of 8.78-61.3% in 17 varieties compared to the control, and decreased in the range of 1.16-26.06% in 14 varieties. Only CS B-17 line showed no variation in this parameter. At 100 mM NaCl concentration, the dry shoot height (DSH) increased by 48% and 7.7%, respectively, compared to the control, in the Sezener-76 and CS B-16. In all other varieties, a decrease in DSH was found in the range of 6.64-39.5%, and in CSLS and parental forms – in the range of 4.41-58.8%. In terms of dry shoot weight (DSW), Tashkent-1 showed the minimum (5.49%) and Kyrgyzstan-174 showed the maximum (65.6%) increase compared to the control.

During the study, it was found that the FSH decreased in all samples at a concentration of 200 mM NaCl salt, unlike at a concentration of 100 mM, and this decrease was in the range of 0.86-47.6% for varieties and 2.2-48.9% for CSLs. In terms of fresh root height (FRH), a decrease was observed in all varieties, except for the local Barakat, Zafar, Ganja-182 and the introduced Tashkent-3, Assos, Cristina varieties. The FRH increased only in 2 CSLs, while the other 13 CSLs showed decreasing in the range of 3.94-47.8%, corresponding to the double haploid Pima 3-79 genotype. During the study, at a concentration of 200 mM NaCl salt, only a decrease was found in the fresh root weight (FRW), unlike at a concentration of 100 mM salt. The highest decrease among the varieties was determined in the local Karabakh-11 (51.6%), and among the CSLs in CS B-18 (50.4%). In this concentration of NaCl, an increase in the FRW was observed in the local Barakat, introduced May-344, PG, CS B-05 genotypes. At a concentration of 200 mM NaCl, the DSH increased only in the Navai-9 and CS B-16 (2.5% and 2.6%, respectively) which differed from all other genotypes. The DSW of the genotypes decreased only at high salt concentration, unlike at 100 mM concentration, and this decrease was in the range of 0.88-68% for varieties, and 10-56.6% for lines. According to the dry root weight (DRW), an increase in this parameter was determined in genotypes Barakat, Karabakh-12, Ganja-160, Ganja-182, Assos, Select, Flash, May-344, Cristina, Navai-9, Lima, CS B-05 and CS B-16 at 200 mM NaCl concentration. Other CS B lines showed a decrease as a parental forms, and this decrease was in the range of 3.57-81.7%. The decrease of DRW of varieties was found in the range of 5.56-48%.

During the study, both an increase and a decrease of studied morphometric parameters were detected among the genotypes at a concentration of 100 mM NaCl, but only a decrease of FSH, FSW, DSW was observed at a concentration of 200 mM salt. Also, under concentration of 200 mM NaCl, only a decrease in the total fresh, dry mass, and height was found for all samples, unlike at a concentration of 100 mM.

A t-test was performed to determine the presence of a significant difference between the mean values of the groups. It was found that there was a significant difference in the changes in the parameters of total fresh height (TFH), total fresh weight (TFW), total dry height (TDH) and total dry weight (TDW) (Table 4.3.1).

Table 4.3.1.
Average values and coefficient of variation of general morphometric parameters

	TFH (cm)			TFW (mg)			TDH (cm)			TDW (mg)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Mean	25.9	22.4	18.2	838.0	819.3	587.6	22.5	19.6	16.2	198.7	196.7	142.9
SD	3.0	3.2	2.6	460.3	340.9	255.2	2.8	3.2	2.7	87.1	94.1	56.1
CV (%)	11.5	14.4	14.5	54.9	41.6	43.4	12.5	16.5	16.4	43.8	47.8	39.2
t-test	61,696**			23,804**			59,896**			25,546**		

** Significant difference at $p < 0.01$

The change in the coefficient of variation (CV) in the control variant was determined from 11.5 (TFH) to 54.9 (TFW). Also, under stress conditions, TFH and TDH increased at both, TDW increased at 100 mM concentrations of NaCl, while TFW decreased at both stress conditions.

Based on the overall score of morphometric indicators, cluster analysis was performed to identify resistant and susceptible genotypes, and genotypes with complex positive traits were selected.

The varieties studied at a concentration of 200 mM NaCl were concentrated in 4 large groups (Figure 4.3.1), with Navai-9, which was included in the first group, being evaluated as highly resistant and having the least decrease of the morphometric parameter. Samples collected in subcluster 2A had the lowest values for changes in TFW and TDW parameters, while plants collected in subcluster 2B had low changes in morphometric indicators overall, and were evaluated as a tolerant group within the cluster. Genotypes included in subclusters 2C and 2D received low scores for TDW, however subcluster 2C was evaluated as a more sensitive group than subcluster 2D, with a higher reduction TFH and TDH within the cluster.

Samples collected in cluster III were considered to be moderately salt-resistant, occupying an intermediate position in terms of changes in morphometric indicators, while varieties collected in cluster IV were found as sensitive genotypes at high salt concentrations and had a higher decrease in overall morphometric indicators.

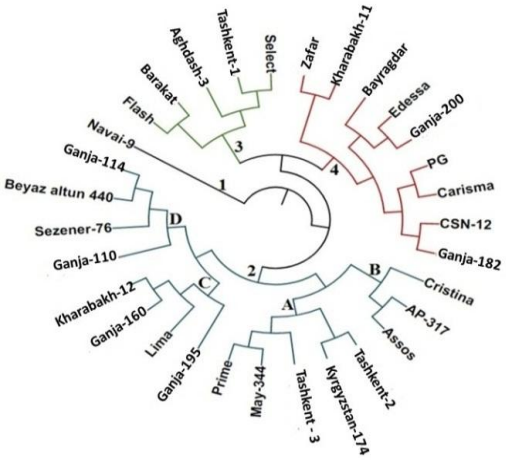


Figure 4.3.1. Cluster analysis of cotton varieties based on morphometric indicators under stress conditions of 200 mM NaCl

As a result of the evaluation of the resistance of CSLs and their donor parents to 200 mM NaCl concentration, group I, which included the parental TM-1, was evaluated as the resistant group, having the lowest change in total morphometric parameters compared to the control (Figure 4.3.2).

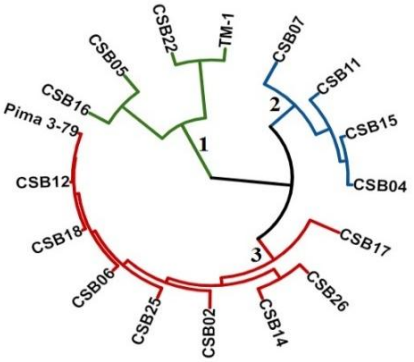


Figure 4.3.2. Cluster analysis of cotton lines and parental forms based on total morphometric parameters under 100 mM NaCl salt stress conditions

It was found that genotypes collected in cluster III had a higher reduction value of the studied morphometric parameters than cluster II, they were evaluated as salt-sensitive, while the lines collected in cluster II were evaluated as moderately salt-resistant genotypes.

During the complex evaluation of the morphophysiological parameters studied for the selection of salt-resistant genotypes, AP-317 among the local varieties, Navai-9, Tashkent-2, Tashkent-3 of Uzbekistan origin, Beyaz altun-440 of Turkey origin and Kyrgyzstan-174 of Kyrgyzstan origin among the introduced varieties, CS B-05, CS B-16, CS B-11, CS B-22 and TM-1 genotypes among CSLs and their parents were evaluated as resistant genotypes, showing a minimum decrease in morphophysiological indicators under the studied high-concentration stress of NaCl. Also, for the first time, during the analysis of the change in expression patterns compared to the control variant under stress conditions, both a decrease and an increase in the expression of the 13 genes studied were observed in the resistant and sensitive genotypes selected according to morphophysiological parameters (Figure 4.3.3).

Thus, based on the data obtained, similar and different levels of transcripts in samples belonging to different geographical groups and differing in salt tolerance indicate that salt tolerance has complex genetic control and such diversity in resistant varieties indicates that resistance has an individual potential level.

CHAPTER V. EVALUATION OF SALT TOLERANCE OF COTTON GENOTYPES BASED ON TOLERANCE INDICES AND GENETIC PARAMETERS

5.1. Evaluation of varieties and lines based on tolerance indices under 100 mM NaCl stress. As a result of the correlation analysis of the tolerance indices of the varieties under normal and stress conditions, a statistically significant ($P \leq 0.01$) correlation was found between STI ($r \sim 0.915^{**}$), MP ($r \sim 0.890^{**}$), YI ($r \sim 0.665^{**}$) and GMP ($r \sim 0.901^{**}$) with potential yield (Yp), and a statistically significant ($P \leq 0.01$) correlation with all tolerance indices with yield under stress conditions (Ys). A negative correlation was found between Ys and the TOL index ($r \sim -0.619^{**}$) and the SSPI index ($r \sim -0.620^{**}$), and a positive correlation was found between Ys and the other parameters.

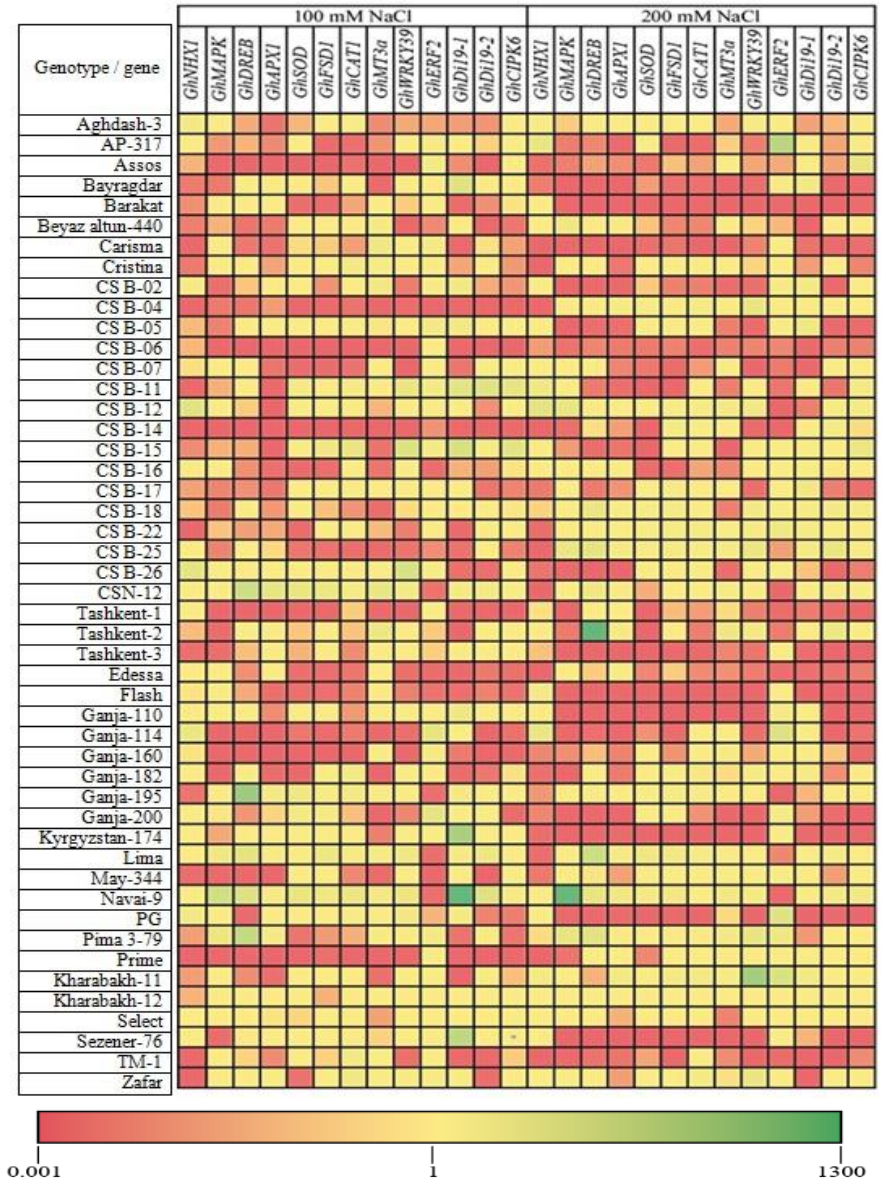


Figure 4.3.3. Heatmap of gene expression (Green and yellow indicate high and low values of increased expression, amber and red indicate decreased expression)

Under the stress of 100 mM NaCl concentration, the highest value of the MSTI parameter was determined in Karabakh-11 (1.61), and the highest values of the STI, MP, YI and GMP parameters were determined in the Ganja-110 variety (2.9, 0.335, 1.712, 0.335, respectively). The lowest SSI index value was recorded in the Navai-9 variety (-2.64), while the TOL and SSPI indices were recorded in the Ganja-160 and Karabakh-11 varieties (-0.092 and -26.562).

As a result of the correlation analysis of the tolerance indices of CSLs and parental forms under stress conditions with 100 mM NaCl concentration, it was found that there was a highly significant positive ($P \leq 0.01$) correlation between STI (r~0.805**), MP (r~0.901**), YI (r~0.675**) and GMP (r~-0.854**) with Yp, and a highly significant positive ($P \leq 0.01$) correlation between STI (r~0.973**), MP (r~0.929**), YI (r~1.000**), GMP (r~0.958**) with Ys, and a significant negative ($P \leq 0.05$) correlation between TOL (r~-0.552*), SSPI (r~-0.552*) and Ys indices.

Under 100 mM NaCl stress, the highest values of STI, MP, YI and GMP indices for CSLs and their parents were determined in the double haploid Pima 3-79 (11.091, 0.66, 3.674, 0.656, respectively), and the MSTI index was determined in the CS-B 11 (1.493). Among the tolerance indices, the lowest values of SSI index were determined in CS-B 11 (-2.511), TOL and SSPI indices were determined in the parental Pima 3-79 genotype (-0.129 and -26.308).

5.2. Evaluation of varieties and lines based on tolerance indices under 200 mM NaCl salt stress. Under the stress condition of 200 mM NaCl, a highly significant positive correlation was found between the STI (r~0.934**), MP (r~0.957**), YI (r~0.845**), GMP (r~0.940**) indices and Yp. In addition a highly significant ($P \leq 0.01$) positive correlation was found between the STI (r~0.953**), MP (r~0.964**), YI (r~1.000**), GMP (r~0.976**), MSTI (r~0.634**) indices and Ys, and a highly significant ($P \leq 0.01$) negative correlation was found between the SSI (r~-0.625**) index and TOL (r~-0.435**) and SSPI (r~-0.434**) indices.

Under stress condition of this concentration, the lowest value of SSI index was determined in Tashkent-2 (0.124), and the lowest values of TOL and SSPI indices were determined in Beyaz altun-440

(0.003 and 0.982, respectively). The highest values of STI, MP, YI and GMP indices were determined in Ganja-110 (5.314, 0.316, 2.18, 0.315, respectively), and MSTI in Tashkent-2 and Beyaz altun-440 varieties (0.974).

As a result of correlation analysis of tolerance indices of CSLs and parental forms under stress of 200 mM NaCl concentration, it was found that there was a statistically significant ($P \leq 0.01$) positive correlation between yield in both normal and stress conditions and all other studied indices, except for SSI and MSTI. During the analysis of tolerance indices, the lowest values of SSI, TOL, SSPI were observed in the CS B-07 (0.278, 0.019 and 3.936, respectively). The highest value of MSTI index was found in the CS B-07 (0.896), and the highest values of STI, MP, YI and GMP indices were found in the double haploid Pima 3-79 genotype (8.159, 0.46, 2.109, 0.439, respectively).

In the current study, for the first time, a highly significant ($P \leq 0.01$) correlation was found between potential yield and yield under stress conditions in both cotton varieties and CSLs with parental forms as a result of correlation analysis of tolerance indices. It was also determined that there was a statistically significant ($P \leq 0.01$) positive correlation between productivity under potential and stress conditions and STI, MP, YI, and GMP indices in all cases, making these indices suitable for identifying salt-tolerant genotypes in cotton.

5.3. Clustering of genotypes based on Principal Component Analysis (PCA). To verify the suitability of factor analysis for the data set, Kaiser-Mayer-Olkin (KMO) sampling adequacy and Bartlett measure were applied for all samples at both control and NaCl concentrations and the results were considered acceptable.

Table 5.3.1.

KMO and Bartlett measure

Test		0 mM NaCl	100 mM NaCl	200 mM NaCl
Kaiser-Mayer-Olkin measure of sampling adequacy		0.736	0.784	0.807
Bartlett`s sphericity criterion	Approximately χ-square	776,906	865.243	940.313
	Degrees of freedom	171	171	171
	Significance	0	0	0

At the same time, as a result of PCA, 5 components were identified in the control variant (Figure 5.3.1), and 4 components with eigenvalues above 1 were identified among the 19 different components studied at both 100 mM (Figure 5.3.2) and 200 mM NaCl concentrations (Figure 5.3.3).

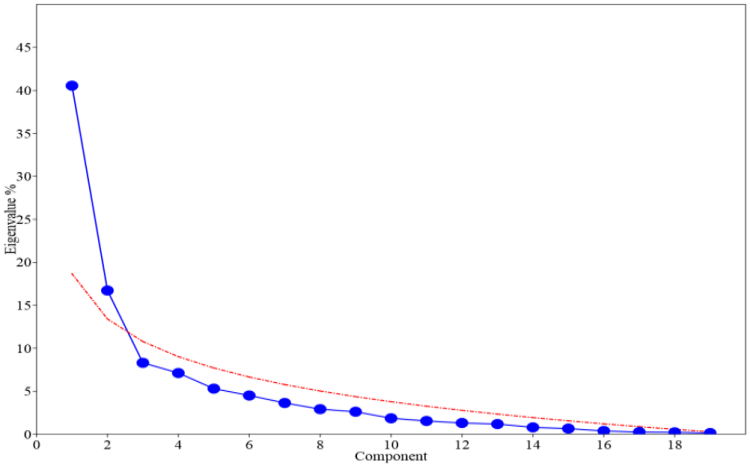


Figure 5.3.1. Eigenvalue plot of components for the control variant of cotton genotypes

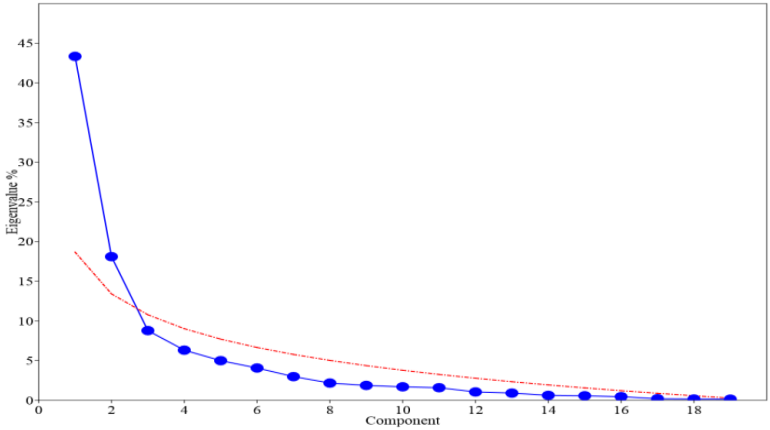


Figure 5.3.2. Eigenvalue plot of components of cotton genotypes at 100 mM NaCl concentration

It was found that the first 5 components accounted for 78.007% of the total variation in the control variant, 4 components with eigenvalues above 1 accounted for 76.547% of the total variation in the 100 mM, and 79.758% in the 200 mM concentration of NaCl.

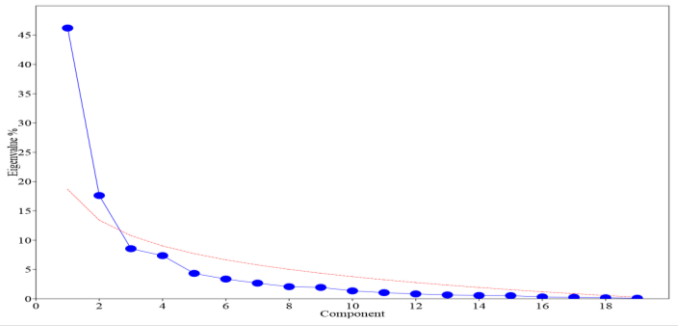


Figure 5.3.3. Eigenvalue plot of components of cotton genotypes at 200 mM NaCl salt concentration

In the study, genotypes that were close to each other were grouped according to the similar values observed for the components shown in the plot, and it was found that both varieties and lines exhibited great variability in morphophysiological and genetic traits under the control (Figure 5.3.4) and the two different concentrations of NaCl (Figure 5.3.5 and Figure 5.3.6).

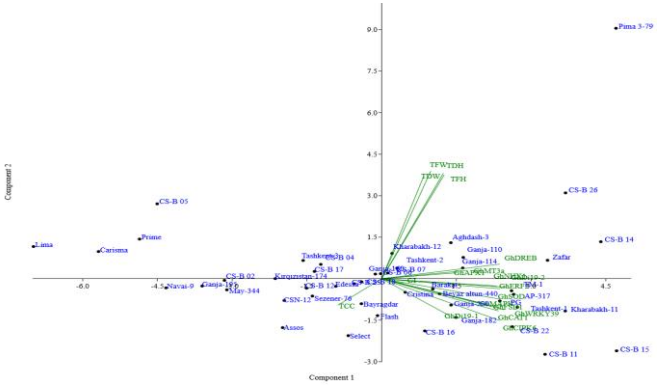


Figure 5.3.4. PCA analysis of cotton genotypes in the control variant

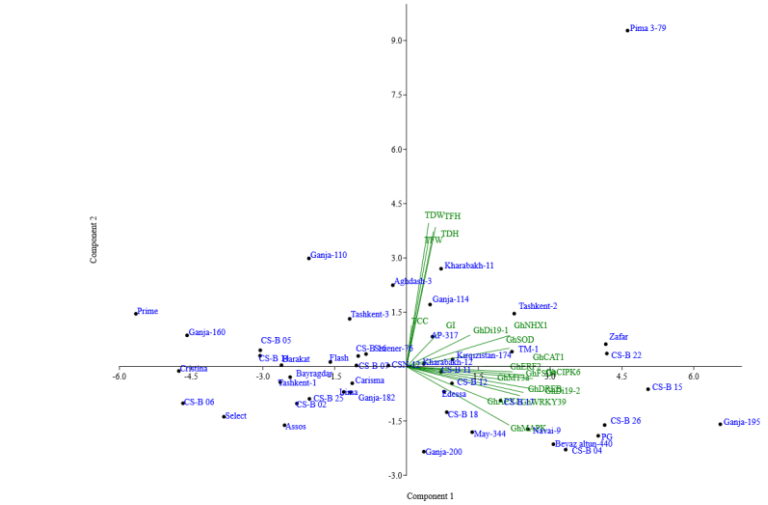


Figure 5.3.5. PCA analysis of cotton genotypes at 100 mM NaCl concentration

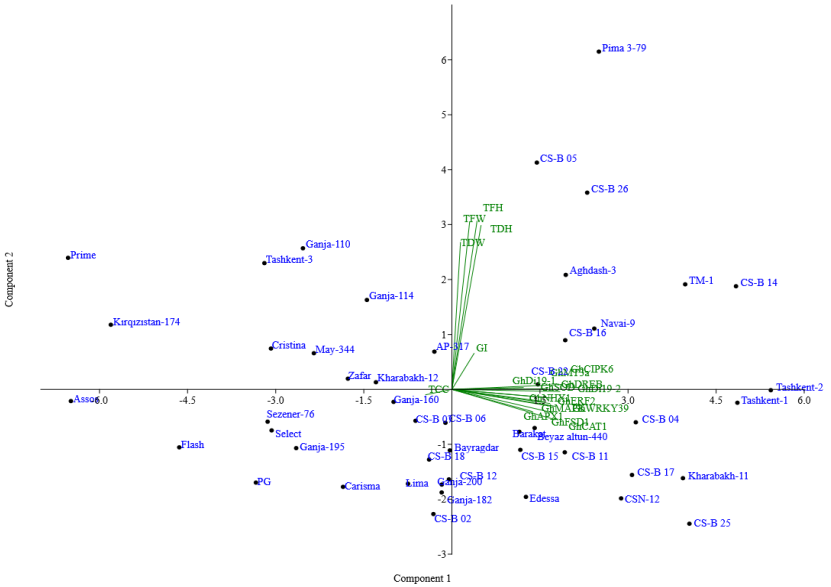


Figure 5.3.6. PCA analysis of cotton genotypes at 200 mM NaCl concentration

As a result of PCA, it was found that while some components showed slight depression in both varieties and CSLs and their parents, others exhibited a wide degree of depression and were included in different groups based on the level of resistance, genotypes, and all parameters measured.

RESULTS

1. As a result of comparative analysis of transcriptome data, a significant ($P \leq 0.05$) difference was found in the expression level of the *GhDi19-1* gene between the control and 100 mM NaCl salt, the expression level of the *GhNHX1*, *GhDREB*, *GhAPX1*, *GhFSD1*, *GhERF2*, *GhDi19-2* and *GhCIPK6* genes between the control and 200 mM NaCl salt, and the expression level of the *GhSOD*, *GhCAT1* and *GhMT3a* genes between the control and both concentration stress conditions.

2. CSLs showed a wide variation in gene expression levels, and the levels of different genes were both similar and different from the parental forms. The change in the expression level of the *GhAPX1* gene in the parental Pima 3-79 genotype was not similar to any line.

3. A significant relationship was found between salt concentration and germination parameters at the 99% probability level, and an increase in the dose of stress caused a significant change in these indicators. Although both an increase and a decrease in the total chlorophyll content were observed in different genotypes during the first 4 weeks under stress conditions, a decrease in the total chlorophyll content was determined in all genotypes under stress with a concentration of 200 mM NaCl 5 weeks after the stress treatment.

4. Although both an increase and a decrease in the total dry and wet height and weight indicators of plants were detected under a concentration of 100 mM NaCl, only a decrease in these indicators was determined at a concentration of 200 mM NaCl.

5. It was found that there was a statistically significant ($P \leq 0.01$) positive correlation between the potential and stress yield and between the STI, MP, YI and GMP indices in cotton varieties, also in chromosome substitution lines and their parental forms under both

100 mM and 200 mM NaCl salt stress.

6. A wide variation was found in the expression levels of the 13 genes studied in both resistant and susceptible genotypes identified based on morphophysiological parameters, and both increases and decreases in transcript levels were observed within the group. Among the genes studied, only a decrease in the expression level of the *GhMT3a* gene was observed in susceptible varieties and lines compared to the control.

7. The principal component analysis confirms that the study of the total fresh height, total fresh weight, total dry height, and total dry weight parameters at 100 mM and 200 mM concentration of NaCl salt is significant, accounting for 76.6 and 79.8% of the total variation, respectively.

RECOMMENDATIONS

1. Gene-specific primers designed in the research work can be used to identify relevant genes and assess their expression levels.

2. It is recommended to use the local AP-317, introduced Navai-9, Tashkent-2, Tashkent-3, Beyaz altun-440 and Kyrgyzstan-174 genotypes, which have been selected resistant under high salt concentrations, as parental forms in selection programs for salt tolerance in cotton.

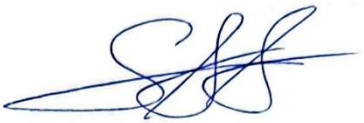
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17. Ализаде, Ш., Алиева, К. Оценка уровня экспрессии гена *GhNHX1* у генотипов хлопчатника в условиях солевого стресса // – Baku: VIII International scientific conference of young researchers Dedicated to the 101th Anniversary of the National leader of Azerbaijan, Heydar Aliyev, – 2024. – p.188-189.

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The defence will be held on 01 December 2026 at 11:00 at the meeting of the Dissertation council FD 1.37 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

Address: AZ 1106, Baku, Azadlig Ave 155.

Dissertation is accessible at the Library of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

Electronic version of abstract is available on the official website of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan (https://genres.az/az).

Abstract was sent to the required addresses on 29 October 2026.

Signed for print: 26.10.2026

Paper format: A5

Volume: 35 070

Number of hard copies: 20