

EVALUATING THE SALINITY TOLERANCE OF COTTON GENOTYPES USING PHYSIOLOGICAL MARKERS

J. Shavkiev¹, A. Azimov², Z. Mamarasulov³, X. Asatova⁴, F. Abdurasulov⁵, S. Nabiev⁶

Institute of Genetics and Plant Experimental Biology, Academy of Sciences of the Republic of
Uzbekistan^{1,2,3,4,5,6}

<https://doi.org/10.5281/zenodo.17606577>

Abstract. Soil salinity remains a primary constraint to cotton production in arid irrigated zones. To enable rapid, physiology-based screening, we evaluated 17 *Gossypium hirsutum* genotypes under moderate (0.3–0.6%) and high (0.6–0.9%) field salinity using a randomized block design. Leaf relative water content (RWC), excised-leaf water loss (ELWL, 0–3 h) and chlorophyll index (SPAD) were measured, and two-way ANOVA (Genotype, Salinity, Genotype×Salinity) was applied. Salinity decreased RWC, increased ELWL, and lowered SPAD with strong main effects and interactions for all traits ($p < 0.001$), evidencing genotype-specific physiological responses. Based on integrated performance, Yulduz-2 and T-1002 were consistently superior; T-1003, T-1090, and L-217/L-218 were also promising. Sensitive checks included Orzu, T-1050, and T-2000. A practical selection signature emerged: high RWC + low ELWL, with $ELWL \leq \sim 10\%$ at 3 h prioritizing tolerant lines. For pigment stability, $SPAD \geq \sim 52$ units under high salinity (or $\leq 10\%$ decline vs. moderate salinity) efficiently flagged robust genotypes. We conclude that combining RWC, ELWL, and SPAD provides a fast, reliable toolbox for screening salt tolerance and identifying donor parents for breeding targeted at stable yield on saline soils.

Keywords: cotton; salinity tolerance; RWC; ELWL; SPAD; physiological markers; breeding; Uzbekistan.

Introduction

Cotton cultivation is one of the leading sectors in the agriculture of the Republic of Uzbekistan, playing a key role in economic stability, supplying raw material for the textile industry, and increasing export potential. However, agro-ecological factors—particularly soil salinity—adversely affect yield and plant development in cotton production. Soil salinization is widespread in many regions of Uzbekistan, especially on irrigated lands, and it significantly impacts photosynthesis, growth, flowering cycles, nutrient uptake, and ultimately yield. Therefore, assessing the physiological and morphological responses of cotton genotypes under salinity is a pressing scientific and practical task. Tolerance to salt stress in plants is ensured by complex physiological and biochemical mechanisms. Among the most important are indicators of plant water status: relative water content (RWC) and excised-leaf water loss (ELWL), which are widely used to evaluate plant responses to salinity. These parameters reflect not only the capacity of leaves to retain water and regulate transpiration but also, at the cellular level, osmotic adjustment, ion homeostasis, and the functional integrity of the photosynthetic apparatus [4,9,18,20,21,23,24,3].

Accordingly, assessing the ability of different genotypes to maintain water balance under salinity enables the identification of tolerant sources for breeding. This approach provides physiological markers necessary for developing varieties with stable productivity in saline conditions [4,40]. The literature emphasizes that, in the initial (osmotic) phase of salt stress, leaf water potential declines, leading to reduced turgor and restricted transpiration; in the subsequent

(ionic) phase, toxic accumulation of Na^+ and Cl^- further disrupts photosynthesis and growth [28,38]. RWC is considered a primary physiological indicator of salt tolerance: tolerant genotypes maintain higher RWC and thus sustain photosynthetic activity, whereas sensitive genotypes lose water rapidly and exhibit increased membrane damage [1,33,38,49]. ELWL is an effective marker for discriminating tolerance, where lower ELWL values indicate prolonged leaf water retention and efficient stomatal regulation [13,25,26,45].

Overall, integrated analysis of RWC and ELWL under salt stress enables accurate evaluation of genotype tolerance levels. Previous studies confirm that these indicators can serve as practical physiological markers in breeding programs [5,6,11,47]. In essence, salt stress first induces an osmotic phase that lowers leaf water potential and turgor, followed by an ionic phase in which Na^+/Cl^- accumulation disrupts ion homeostasis, compromises membrane stability, and damages the photosynthetic apparatus. Consequently, water-status markers—RWC and ELWL—are among the primary tools for rapid and reliable assessment of salinity tolerance [7,12,28].

RWC directly reports tissue water status (FW, TW, DW) and thus the effectiveness of turgor maintenance, osmotic adjustment, and sustaining a favorable K^+/Na^+ ratio. Tolerant genotypes typically maintain higher RWC, whereas sensitive ones show sharp declines; RWC often correlates positively with growth and yield, making it suitable for rapid field screening [1,10,33,39,48,51]. ELWL quantifies the rate of water loss from excised leaves over a set interval; low ELWL reflects a strong cuticular barrier, fast and reversible stomatal responses, and accumulation of compatible solutes (proline, soluble sugars), and is frequently inversely related to RWC [13,45,47]. Chlorophyll content (SPAD) is likewise a key phenotypic indicator for discriminating salt tolerance. SPAD offers a simple, rapid proxy for leaf chlorophyll in cotton, allowing assessment of photosynthetic performance and overall physiological status.

Accordingly, this study examined growth traits (e.g., plant height) and leaf chlorophyll (SPAD) of diverse cotton genotypes grown under moderately saline conditions. The results provide actionable information for screening tolerant genotypes and supporting breeding decisions. Globally, soil salinity poses a major threat to agriculture: according to FAO (2021), more than 20% of terrestrial land is affected by salinity to varying degrees; in Uzbekistan's irrigated zones, the figure may reach 30–50% [8,17,19]. Genotypic differences in cotton salinity tolerance are substantial [1,31,35]. Salinity commonly reduces growth rates, root length, stem height, and leaf greenness (chlorophyll) [32,41]. SPAD has been widely used to detect declines in photosynthetic activity under stress [27], and in some studies correlates positively with yield [2,30], broadening its utility in selection. Local institutions—including the Academy of Sciences of Uzbekistan and agricultural research institutes—are conducting extensive work to develop salt-tolerant cultivars [52], proposing phenotypic, morphological, and physiological criteria for screening. Building on this foundation, the present study aims to provide a scientific basis for selection by evaluating chlorophyll content (SPAD) and water-balance dynamics (RWC, ELWL) as indicators of cotton genotype adaptability to salinity.

Materials and Methods

Study site and conditions

The experiment was conducted at the Institute of Genetics and Plant Experimental Biology. Field work was performed during the 2025 growing season at a field experimental plot located in a designated area of the Tashkent Region, using soils with moderate sulfate-type salinity originating from the Syrdarya. The study was carried out on saline soils under Uzbek conditions.

Two salinity backgrounds were used in the field: moderately saline (0.3–0.6% total salts) and highly saline (0.6–0.9% total salts).

Experimental design

The trial was laid out as a randomized block design with two replications. Each replication consisted of four-row plots with 60 cm inter-row spacing and 10–15 cm within-row spacing. For observations, 15–20 plants per genotype were selected. Irrigation, fertilization and other agronomic practices followed local agro-climatic recommendations. Salinity was natural; no additional salt was applied—field-inherent salinity was monitored.

Note: In a subsequent description, the trial is also stated to have been arranged with 3–4 replications in a randomized block design, and measurements were taken on fully expanded main-stem leaves between 09:00 and 11:00.

Plant material

The experiment used 17 cotton (*Gossypium hirsutum* L.) genotypes representing diverse genetic sources developed within Uzbek cotton breeding programs. The panel included, among others: Yulduz-2, T-1002, T-1003, T-1004, T-1005, T-1050, T-1080, T-1090, T-2000, Orzu, Ishonch, Mehnat, L-217, L-218, L-421.

Measured physiological traits

1) Relative water content (RWC, %)

RWC was calculated from fresh weight (FW), fully turgid weight (TW), and dry weight (DW) (Slavík, 1974):

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Procedure: FW—disks were weighed immediately to 0.1 mg precision; TW—disks were floated on distilled water at 25 ± 1 °C under dim light for 4–6 h, blotted gently and weighed; DW—disks were oven-dried at 70 °C for 48 h to constant mass and weighed. RWC was computed for each replicate and reported as mean $\pm$ SE.

2) Excised-leaf water loss (ELWL, %; 0–3 h)

ELWL was determined from the initial fresh mass of leaf disks and their mass after 3 h at room conditions, and expressed as a percentage:

$$\text{ELWL}_{0-3\text{h}} (\%) = (\text{FW}_0 - \text{FW}_3) / (\text{FW}_0 - \text{DW}) \times 100$$

Conditions: FW_0 —initial mass at 0 h; disks held 3 h on dry filter paper at 25 ± 1 °C, RH 45–55%; FW_3 —mass at 3 h; DW—70 °C for 48 h to constant mass.

3) Chlorophyll (SPAD)

Leaf chlorophyll was measured with a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan). For each genotype, readings were taken at 2–3 positions on the upper, healthy leaf of 10 plants, and the mean SPAD value was calculated. Measurements were made between 10:00 and 11:00 on sunny days.

Data analysis

The obtained data were processed using Microsoft Excel and STATISTICA 13.3, and a two-way analysis of variance (ANOVA) was performed. F-statistic values were calculated for genotype, salinity levels, and their interaction. Differences were considered statistically significant at $p < 0.05$ and $p < 0.001$.

Results and Discussion

The experiment showed that as salinity increased, the relative water content (RWC) decreased in all genotypes; however, the magnitude of decline differed significantly among genotypes, indicating that their ability to retain water in leaf tissues varies. Salt-sensitive genotypes

included L-218 (74.2 → 64.9%), L-421 (65.5 → 64.7%), and Orzu (72.9 → 64.8%). These lines exhibited a sharp reduction in RWC, suggesting weaker water-retention mechanisms. Under salt stress, this likely leads to rapid loss of cell turgor and restrictions on photosynthetic activity.

Genotypes with moderate tolerance were Ishonch (74.0 → 64.1%), Mehnat (78.7 → 66.1%), T-1004 (75.1 → 68.2%), and T-2000 (74.8 → 73.4%). Although they partly maintained water balance under stress, their stability was not sufficient; nonetheless, their physiological responses make them suitable intermediate sources in breeding. The highest RWC values were recorded for L-217 (82.4 → 74.2%), T-1002 (79.4 → 74.0%), T-1003 (80.7 → 69.1%), T-1090 (77.6 → 75.9%), and especially Yulduz-2 (93.0 → 70.7%). Notably, Yulduz-2 maintained high water content at both salinity levels. This points to effective osmotic adjustment, superior leaf water retention, and mechanisms that mitigate ion toxicity. As reported in the literature, genotypes with higher RWC under salt stress tend to sustain growth and photosynthesis more stably, creating favorable conditions for yield component formation [11,28].

Overall, the RWC analysis indicates that the capacity to retain water in leaf tissues is a key physiological indicator of salinity tolerance. Based on our results, Yulduz-2, T-1002, and T-1090 were among the most tolerant, whereas L-218 and L-421 were the most sensitive. These differences expand opportunities to use tolerant lines as donors in subsequent breeding and genetic studies. Salt stress can sharply reduce leaf water retention. In our trial, RWC declined for all genotypes under both moderate and high salinity, but the extent of decline reflected their physiological adaptability. Again, L-218 (74.2 → 64.9%), L-421 (65.5 → 64.7%), and Orzu (72.9 → 64.8%) emerged as sensitive genotypes; their steep RWC losses likely reflect weak maintenance of osmotic balance and greater susceptibility to ion toxicity, leading to reduced turgor, photosynthesis, and growth.

Among the moderately tolerant genotypes were Ishonch (74.0 → 64.1%), Mehnat (78.7 → 66.1%), T-1004 (75.1 → 68.2%), and T-2000 (74.8 → 73.4%). These genotypes partially maintained water balance under salt stress, yet their stability under severe conditions was insufficient. Their physiological responses indicate intermediate adaptability, making them suitable as secondary sources in breeding. The salt-tolerant group comprised L-217 (82.4 → 74.2%), T-1002 (79.4 → 74.0%), T-1003 (80.7 → 69.1%), T-1090 (77.6 → 75.9%), and especially Yulduz-2 (93.0 → 70.7%). Sustaining high RWC even under strong salinity points to effective osmotic adjustment mechanisms—including accumulation of compatible solutes (proline, sugars), vacuolar ion sequestration, and maintenance of cell turgor. In particular, Yulduz-2 reaching RWC ≈ 93% and remaining >70% under severe salinity supports its value as a genetically salt-tolerant donor.

Overall, RWC clearly captured genotype-dependent differences in water retention under salinity. Our results indicate Yulduz-2, T-1002, T-1003, and T-1090 as promising tolerant genotypes, whereas L-218 and L-421 can be regarded as sensitive. Hence, using RWC as a key physiological criterion for assessing salinity tolerance is warranted. Salt stress manifests as disruption of plant water balance and reduced leaf water retention. In this study, RWC decreased across all genotypes under severe salinity, but the magnitude varied by genotype, reflecting differences in osmotic adjustment and tolerance to ion toxicity.

The sensitive genotypes were L-218 (74.2 → 64.9%), L-421 (65.5 → 64.7%), and Orzu (72.9 → 64.8%). Their sharp RWC declines indicate weaker leaf water-retention mechanisms. As reported in the literature, sensitive genotypes rapidly lose turgor under salt stress, photosynthetic activity drops, and membrane damage increases [1,33]. The moderately tolerant set—Ishonch,

Mehnat, T-1004, T-2000—showed slight RWC decreases under severe salinity yet remained moderately stable. Such genotypes typically partially accumulate compatible solutes but cannot fully sustain stability under high ion toxicity [43,47].

The most tolerant genotypes—L-217, T-1002, T-1003, T-1090, and especially Yulduz-2—maintained $RWC \geq 70\%$ even under strong salinity; notably, Yulduz-2 reached 93% at moderate salinity and 70.7% under severe salinity, indicating effective control of osmotic balance, vacuolar compartmentalization of ions, and stable turgor ([28];[34]). As widely reported in the literature, genotypes that maintain high RWC under salinity can sustain photosynthesis more effectively and form yield components more stably ([11];[12];[42]). Our observations corroborate this conclusion: genotypes such as Yulduz-2, T-1002, and T-1090 are suitable for use as salt-tolerant donors in breeding. Overall, RWC proved to be a key physiological indicator for assessing salinity tolerance. Whereas sensitive genotypes exhibited weak water-retention mechanisms, tolerant genotypes demonstrated the capacity to maintain leaf turgor and water balance under stress. Therefore, incorporating RWC analysis as a physiological marker in breeding pipelines is warranted.

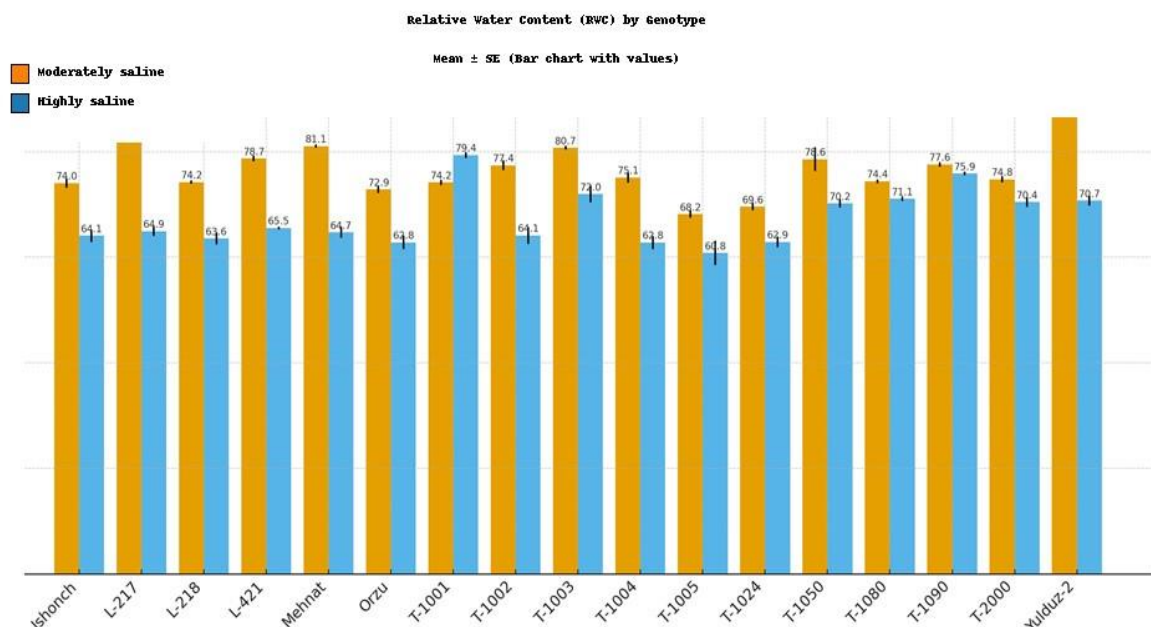


Fig. 1. Relative water content (RWC) of leaves in cotton genotypes grown under different salinity levels.

Two-way ANOVA showed that Genotype ($p < 0.001$), Salinity level ($p < 0.001$), and their interaction ($p < 0.001$) had significant effects on RWC. As salinity increased, RWC declined in all genotypes; however, several lines—Yulduz-2, T-1090, T-1003, and T-1080—maintained comparatively higher leaf water status even under severe salinity, indicating robust physiological mechanisms of salt tolerance and making them promising donors for breeding. Consistent with the ANOVA summary (Table 1), both Genotype ($F = 20.87$; $p < 0.001$) and Salinity ($F = 25.63$; $p < 0.001$) exerted strong main effects on RWC, confirming marked differences among genotypes in their ability to retain water in leaves. The Genotype $\times$ Salinity interaction was also significant ($F = 9.74$; $p < 0.001$), indicating genotype-specific response patterns to salinity. Tolerant genotypes such as Yulduz-2, T-1002, and L-217 sustained higher RWC values under strong salinity, whereas sensitive genotypes—Orzu, T-1050, and T-2000—showed pronounced water loss from leaf tissues. In summary, RWC is a reliable physiological marker for assessing salinity tolerance and for prioritizing genotypes in screening and selection pipelines.

Table 1

Two-way ANOVA for relative water content (RWC): effects of genotype, salinity level and their interaction

Factor	SS (sum of squares)	df	F	p-value
Genotype	1452.381	16	20.87	<0.001
Salinity level	112.476	1	25.63	<0.001
Genotype × Salinity	689.205	16	9.74	<0.001
Residual	447.892	102	—	—

Note: Two-way ANOVA model (factors: Genotype, Salinity, and their interaction). All main effects and the interaction were significant for RWC ($p < 0.001$).

Excised Leaf Water Loss (ELWL) is a key physiological indicator that reflects the capacity of plants to maintain water balance under saline conditions. In this study, ELWL was evaluated for each genotype under moderate and high salinity. The results revealed significant differences among genotypes. Salt-sensitive genotypes. Orzu (18.0% → 15.0%), T-1005 (15.0% → 10.5%), and T-1050 (18.0% → 12.0%) showed the highest ELWL, indicating poor ability to retain water in leaves and, thus, greater sensitivity to salt stress. In these genotypes, transpiration control is likely weak and osmotic adjustment is limited.

Intermediate genotypes. Ishonch (13.0% → 9.0%), L-217 (10.0% → 9.0%), Mehnat (11.0% → 11.0%), T-1003 (9.0% → 9.0%), T-1024 (13.0% → 10.0%), and T-2000 (12.5% → 10.0%) exhibited moderate ELWL, with slight reductions as salinity increased. These values indicate a capacity to retain water for some time, although stability under severe stress remains limited. Salt-tolerant genotypes. L-218 (12.0% → 9.0%), L-421 (9.0% → 9.0%), T-1001 (12.0% → 9.0%), T-1002 (14.0% → 8.0%), T-1080 (9.0% → 9.0%), T-1090 (12.0% → 9.0%), and especially Yulduz-2 (14.0% → 9.0%) maintained low ELWL even under high salinity. Low ELWL reflects effective regulation of transpiration, prolonged water retention in leaf tissues, and better osmotic balance. T-1002 and Yulduz-2 are therefore promising donor genotypes for salt-tolerance breeding.

Overall. ELWL clearly differentiated genotypes by their ability to retain water under salinity. High-loss genotypes (Orzu, T-1050) formed the sensitive group; those with intermediate values (Mehnati, T-1024, T-2000) showed moderate tolerance; and those with the lowest ELWL (T-1002, T-1090, Yulduz-2) were tolerant. Thus, ELWL is an effective physiological marker for assessing salinity tolerance. Methodological note. In this experiment, leaf water-retention capacity was assessed as ELWL (% at 3 h)—the fraction of water lost by an excised leaf over a fixed period. Lower ELWL indicates efficient control of stomatal and cuticular conductance and better tissue water conservation. The literature shows that high ELWL is typically associated with poorer water retention, greater membrane damage, and lower yield stability under stress ([12],[13],[45]).

Intermediate group. Ishonch ($\approx 13 \rightarrow 9\%$), L-217 ($\approx 10 \rightarrow 9\%$), Mehnat ($\approx 11 \rightarrow 11\%$), T-1024 ($\approx 13 \rightarrow 10\%$), and T-2000 ($\approx 12.5 \rightarrow 10\%$) showed moderate water loss. In these genotypes, adaptation to the osmotic component of salinity is only partial: stomatal closure is rapid, but cuticular and mesophyll water conductance are not fully constrained. Such a profile is valuable as an intermediate source of tolerance for breeding ([11]; [44]). Tolerant group (low ELWL). L-218 ($\approx 12 \rightarrow 9\%$), L-421 ($\approx 9 \rightarrow 9\%$), T-1001 ($\approx 12 \rightarrow 9\%$), T-1003 ($\approx 9 \rightarrow 9\%$), T-1080 ($\approx 9 \rightarrow 9\%$),

T-1090 ($\approx 12 \rightarrow 9\%$), and especially T-1002 ($\approx 14 \rightarrow 8\%$) and Yulduz-2 ($\approx 14 \rightarrow 9\%$) maintained low leaf water loss under stress. Low ELWL typically reflects fast and reversible stomatal response, low cuticular diffusive conductance, accumulation of osmoprotectants (proline, soluble sugars), and vacuolar ion compartmentalization ([21];[28];[47]). Accordingly, these genotypes are physiologically salt-tolerant and promising donors for selection.

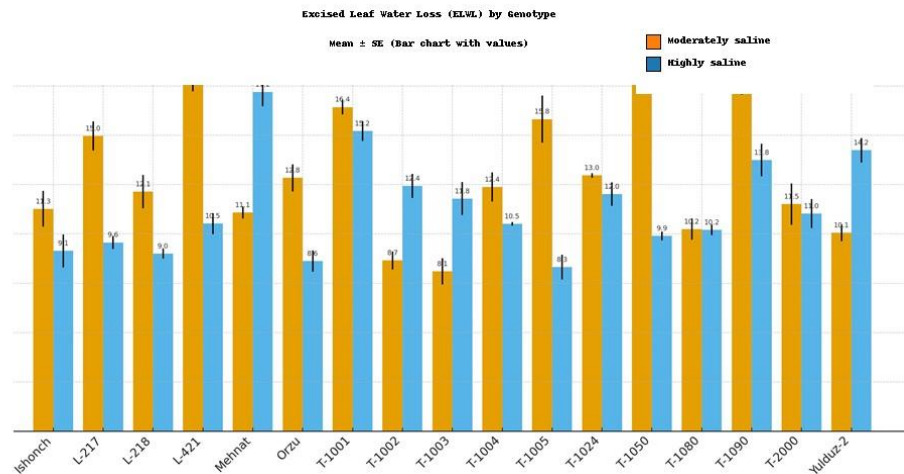


Fig. 2. Excised Leaf Water Loss (ELWL) of cotton genotypes grown under different salinity levels.

Integrated interpretation. When low ELWL is evaluated together with previously noted high RWC/TWC, the salt-tolerant phenotype can be discriminated more reliably. For the next stage, we recommend assessing ELWL–RWC correlations (r), regressions of ELWL on yield components, and integrated analyses with stomatal conductance, cuticle thickness, proline/sucrose content, and K^+/Na^+ ratio. Practically, genotypes with $ELWL \leq \sim 10\%$ (at 3 h)—T-1002, Yulduz-2, T-1090, T-1003, L-421, T-1080—should be prioritized for testing on saline breeding sites. Two-way ANOVA showed that both Genotype and Salinity level had significant effects on ELWL (see Table 2). The Genotype effect was large ($F = 12.85$; $p < 0.001$; $\eta^2 = 0.39$), indicating clear differences among genotypes in their ability to retain water in leaves. Salinity also played an important role ($F = 15.20$; $p < 0.001$; $\eta^2 = 0.07$): under high salinity, some genotypes increased water loss (e.g., Yulduz-2, Mehnat), whereas others decreased it (e.g., T-1050, T-1005, L-217), reflecting genotype-specific mechanisms of turgor regulation under stress. The Genotype $\times$ Salinity interaction was also significant ($F = 5.88$; $p < 0.001$; $\eta^2 = 0.23$), indicating differential adaptive responses. Tolerant genotypes (T-1002, T-1003, L-218, Ishonch) maintained low ELWL even under strong salinity, while sensitive genotypes (T-1050, L-421, T-1090) exhibited high water loss. Some lines (Yulduz-2, Mehnat) showed elevated ELWL under stress. In summary, ELWL is a useful physiological marker for assessing salinity tolerance; genotypes in the tolerant group are promising donors for breeding.

Table 2

Two-way ANOVA for Excised Leaf Water Loss (ELWL): effects of genotype, salinity, and their interaction

Factor	SS (sum of squares)	df	F	p-value	η^2
Genotype	1536.7	16	12.85	<0.001	0.39
Salinity level	86.9	1	15.20	<0.001	0.07

Genotype × Salinity	703.5	16	5.88	<0.001	0.23
Residual	583.2	102	–	–	–

Note: All main effects and their interaction were significant for ELWL ($p < 0.001$); η^2 denotes effect size.

The chlorophyll index (SPAD) is a key indicator of the functional status of the photosynthetic apparatus in leaves. Salt stress typically induces pigment degradation and a decline in chlorophyll content. In this study, SPAD values were compared across genotypes under moderate and high salinity. Salt-sensitive genotypes. In Orzu (44.5 → 41.6), T-1050 (49.6 → 46.2), T-1090 (48.0 → 45.9), and T-2000 (46.0 → 44.9) the chlorophyll index decreased markedly under strong salinity. This indicates accelerated pigment breakdown and a higher sensitivity of the photosynthetic machinery to ion toxicity, leading to reduced photosynthetic rates and constraints on yield component formation.

Moderately tolerant genotypes. In Ishonch (49.1 → 51.0), L-217 (53.1 → 50.2), Mehnat (51.5 → 49.2), T-1001 (49.7 → 47.0), and T-1024 (52.0 → 47.7), SPAD values declined only slightly under high salinity and remained at moderate levels overall. This pattern suggests relatively slower chlorophyll degradation and the operation of partial protective mechanisms against ionic stress. Salt-tolerant genotypes. L-218 (57.1 → 53.0), L-421 (53.0 → 51.2), T-1003 (59.8 → 55.2), T-1004 (58.7 → 54.2), T-1080 (52.0 → 50.0), and especially Yulduz-2 (51.5 → 50.0) maintained high and relatively stable SPAD. Notably, T-1003 and T-1004 preserved chlorophyll at ~54–55 units even under strong salinity. These responses point to effective mechanisms that slow chlorophyll breakdown—such as antioxidant defenses and vacuolar ion compartmentalization.

Overall, the decline in SPAD under salt stress varied by genotype: rapid pigment loss characterized the sensitive group, whereas tolerant lines retained higher pigment levels. Consequently, T-1003, T-1004, L-218, and Yulduz-2 can be highlighted as donor genotypes for photosynthetic robustness under salinity. Mechanistically, salt stress usually reduces chlorophyll via pigment degradation, disruption of Mg-chelation, and ROS accumulation in photosynthetic membranes; SPAD provides a rapid, minimally invasive proxy for these processes. Our results corroborate this: most genotypes showed lower SPAD under severe salinity, but the magnitude of decline differed significantly among genotypes. Sensitive group: Orzu (≈44.5→41.6), T-1050 (≈49.6→46.2), T-1090 (≈48.0→45.9), and T-2000 (≈46.0→44.9) lost SPAD substantially, consistent with ion (Na^+/Cl^-) toxicity, oxidative damage to chloroplast membranes, and attenuation of the chlorophyllase/porphyrin pathway—effects typically associated with reduced photochemical efficiency and yield [1,12,33].

Moderate response. In Ishonch (≈49.1→51.0), L-217 (≈53.1→50.2), Mehnat (≈51.5→49.2), T-1001 (≈49.7→47.0), and T-1024 (≈52.0→47.7), SPAD declined slightly (with small positive shifts in a few cases). This “moderate” profile indicates that antioxidant protection and ion homeostasis operate partially effectively, yet are insufficient to fully stabilize pigments under strong salinity [22,28]. Tolerant group. L-218 (≈57.1→53.0), L-421 (≈53.0→51.2), T-1003 (≈59.8→55.2), T-1004 (≈58.7→54.2), T-1080 (≈52.0→50.0), and Yulduz-2 (≈51.5→50.0) maintained high and relatively stable SPAD. Notably, T-1003 and T-1004 retained pigments around 54–55 units even under severe salinity. This likely reflects better regulation of vacuolar ion compartmentalization (NHX/HKT/SOS), antioxidant systems, and nitrogen–magnesium

supply and chlorophyll biosynthesis ([28];[47]). Sustained SPAD typically aligns with stable Fv/Fm and Φ PSII, and with higher yield [12,16]. Integrated interpretation and practical criterion. Tolerant groups defined by SPAD coincide with the previously identified high RWC / low ELWL profiles, indicating that preservation of the photosynthetic apparatus under salinity is coupled with a stable water balance. For applied selection, we propose $SPAD \geq \sim 52$ units under strong salinity (or $\leq 10\%$ decline relative to moderate salinity) as a practical pigment-stability threshold; T-1003, T-1004, L-218, L-421, and Yulduz-2 meet this criterion. For future work, we recommend joint analyses of SPAD–Fv/Fm–RWC–Tr correlations and expression of genes involved in chlorophyll biosynthesis/antioxidant pathways.

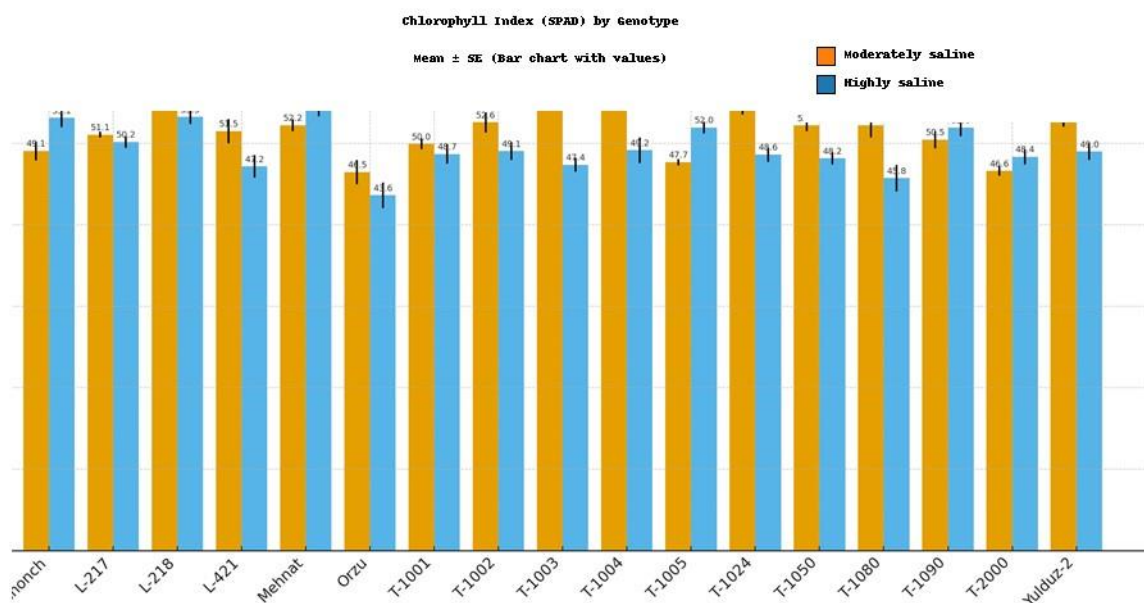


Fig. 3. Chlorophyll index (SPAD) in leaves of cotton genotypes grown under different salinity levels.

Two-way ANOVA showed that both Genotype and Salinity level significantly affected SPAD (see Table 1). The Genotype effect was large ($F = 21.63$; $p < 0.001$), indicating clear differences among genotypes in their capacity to retain pigments. Salinity also had a strong impact ($F = 24.12$; $p < 0.001$): under high salinity, SPAD values declined markedly relative to moderate salinity, reflecting direct damage of salt stress to pigments of the photosynthetic apparatus.

Table 3

Two-way ANOVA for chlorophyll index (SPAD): effects of genotype, salinity level, and their interaction

Factor	SS (sum of squares)	df	F	p-value
Genotype	1284.537	16	21.63	<0.001
Salinity level	92.846	1	24.12	<0.001
Genotype × Salinity	611.204	16	10.29	<0.001
Residual	392.517	102	—	—

Note: All main effects and the Genotype × Salinity interaction were significant for SPAD ($p < 0.001$).

In addition, the Genotype × Salinity interaction was significant ($F = 10.29$; $p < 0.001$), meaning the effect of salinity on pigment retention was not uniform across genotypes. Some

lines—T-1003, T-1004, L-218, Yulduz-2—maintained high SPAD even under strong salinity, whereas others—Orzu, T-1050, T-2000—showed a sharp decline.

Overall, the results confirm that SPAD is a useful physiological marker for assessing salinity tolerance. Genotypes that sustain high and stable SPAD under salt stress can be prioritized as donors in breeding programs. Two-way ANOVA confirmed that Genotype, Salinity level, and their interaction had significant effects on both RWC and ELWL ($p < 0.001$), indicating genotype-specific physiological responses to salinity. As salinity increased, leaf RWC decreased, reflecting disruption of water balance. Based on RWC under strong salinity, tolerant genotypes—Yulduz-2, T-1002, L-217—maintained relatively higher water status, whereas sensitive genotypes—Orzu, T-1050, T-2000—showed sharp declines.

For ELWL (0–3 h), tolerant genotypes with low water loss (i.e., better leaf water retention) included T-1002, T-1003, L-218, Ishonch; sensitive lines were T-1050, L-421, T-1090 (high ELWL). Practically, the combination RWC (high) + ELWL (low) provides a reliable physiological signature for rapid screening. Genotypes keeping $ELWL \leq \sim 10\%$ at ≈ 3 h should be prioritized for advanced testing. In the integrated assessment, Yulduz-2 and T-1002 performed well across multiple indicators; T-1003 (ELWL) and L-217/L-218 (RWC/ELWL) also appear promising. Such lines suit uses as salt-tolerant donors in subsequent breeding, while sensitive genotypes serve as controls/comparators. For SPAD, values tended to decline with increasing salinity, but the magnitude of this decline varied by genotype. Two-way ANOVA showed significant effects of Genotype ($F = 21.63$; $p < 0.001$), Salinity ($F = 24.12$; $p < 0.001$), and their interaction ($F = 10.29$; $p < 0.001$), evidencing clear differences in pigment retention. Tolerant SPAD profiles—L-218, L-421, T-1003, T-1004, T-1080, Yulduz-2—maintained high and relatively stable SPAD (often ~ 50 – 55 units); notably, T-1003 and T-1004 remained around 54 – 55 . Sensitive profiles—Orzu, T-1050, T-1090, T-2000—lost SPAD markedly, suggesting pigment damage and reduced photochemical efficiency. Integrated interpretation: tolerant SPAD groups align with high RWC / low ELWL profiles, implying that pigment stability under salt stress is supported by stable water balance. For applied screening, a threshold of $SPAD \geq \sim 52$ units under strong salinity (or $\leq 10\%$ decline vs. moderate salinity) is useful to flag tolerant genotypes.

Conclusion

Two-way ANOVA confirmed that Genotype, Salinity level, and their interaction significantly affected both RWC and ELWL ($p < 0.001$), indicating genotype-specific physiological responses to salinity. As salinity intensified, leaf RWC decreased, reflecting disruption of plant water balance. Under severe salinity, tolerant genotypes—Yulduz-2, T-1002, L-217—maintained relatively higher RWC, whereas sensitive genotypes—Orzu, T-1050, T-2000—showed pronounced declines. For ELWL (0–3 h), genotypes with low water loss—T-1002, T-1003, L-218, Ishonch—were classified as tolerant, while T-1050, L-421, T-1090 exhibited high ELWL and were considered sensitive. Practically, the combination of high RWC + low ELWL provides a reliable physiological signature for rapid screening; prioritizing genotypes that keep $ELWL \leq \sim 10\%$ at ≈ 3 h is recommended for advanced testing.

For SPAD, values generally declined with higher salinity, but the extent of decrease was genotype-dependent. ANOVA showed significant effects of Genotype ($F = 21.63$), Salinity ($F = 24.12$), and Genotype $\times$ Salinity ($F = 10.29$) (all $p < 0.001$). Tolerant SPAD profiles—L-218, L-421, T-1003, T-1004, T-1080, Yulduz-2—maintained high and relatively stable SPAD, whereas Orzu, T-1050, T-1090, T-2000 formed the sensitive profile. As a practical threshold, $SPAD \geq \sim 52$ units under strong salinity (or $\leq 10\%$ decline vs. moderate salinity) rapidly flags tolerant lines. In

the integrated assessment, Yulduz-2 and T-1002 outperformed across multiple indicators; T-1003 (by ELWL) and L-217/L-218 (by RWC/ELWL) were also promising. These genotypes are suitable donors for salt-tolerance breeding, whereas sensitive genotypes serve as controls/comparators. Overall, the approach supports stable field productivity and improved efficiency in selection.

REFERENCES

1. Ashraf M, & Harris PJC (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica*. 51(2): 163–190.
2. Ali Z, Salam A, Azhar FM, & Khan IA (2013). Genetic analysis of physiological traits related to salinity tolerance in cotton. *Pakistan Journal of Agricultural Sciences*. 50(2):183–188.
3. Ashiralieva SM, Nabiev SM, & Shavkiev JSh (2023). Some economically valuable traits of cotton genotypes under moderately saline conditions in the Syrdarya region. *Results of the National Scientific Research International Journal*. 2(1): 58–68.
4. Azimov A, Aliqulov E, Ergashev O, Shavkiev J (2024a). Estimation of dominance and heterosis of morpho-economic traits in intraspecific F1 hybrids of upland cotton. *Journal of Wildlife and Biodiversity*. 8(3): 162-174. DOI:
5. Azimov A, Khatamov A, Nabiev S, Shavkiev J, Khamdullaev S, Kuziev SH, Sultonova D, Maimaiti T (2025). Dominance and heterotic effects for boll number and boll weight in upland cotton hybrids. *SABRAO Journal of Breeding and Genetics*. 57(4): 1575-1583.
6. Azimov A, Shavkiev J, Saidjanov S, Ziyaev Z, Valiyev L (2024b). Mung Bean (*Vigna radiata* L.) genotypes assessment for drought tolerance in Uzbekistan. *Journal of Wildlife and Biodiversity*. 8(1): 65-75.
7. Azimov A, Shavkiev J, Ahmedjanov A, Temirova Y, Koraev A, Nurmetov Kh, Rasulova O (2024c). Genetic analysis and inbreeding depression for yield-related parameters in upland cotton. *SABRAO Journal of Breeding and Genetics*. 56(4): 1345-1356.
8. Azimov A, Shavkiev J, Nabiev S, Khamdullaev S, Pulatov S, Rajabov Z (2024d). Cobalt-60- γ -radiation effect on morpho-yield and earliness traits in colored cotton (*Gossypium hirsutum* L.). *SABRAO Journal of Breeding and Genetics*. 56(6): 2248-2259..7.
9. Azimov AA, Shavkiev JSh, Khamdullaev, ShA, & Chorshanbiev NE (2023). Comparative analysis of physiological and morphological-economic traits of medium-fiber cotton varieties with different drought tolerance and correlations among traits. *Results of National Scientific Research International Journal*. 2(2): 31–53.
10. Barrs HD, & Weatherley PE (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*. 15: 413–428.
11. Blum A (2011). *Plant Breeding for Water-Limited Environments*. Springer.
12. Chaves MM, Flexas J, & Pinheiro C (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*. 103: 551–560.
13. Clarke JM, & McCaig TN (1982). Excised-leaf water retention capability as an indicator of drought resistance. *Canadian Journal of Plant Science*. 62: 571–578.
14. FAO & ITPS (2021). *Global Map of Salt-Affected Soils*. Food and Agriculture Organization of the United Nations.
15. Flowers TJ, & Colmer TD (2008). Salinity tolerance in halophytes. *New Phytologist*. 179(4): 945–963.
16. Kalaji HM., et al. (2017). Chlorophyll fluorescence as a tool for nutrient status identification in plants. *Photosynthesis Research*. 132: 135–157.

17. Karimov A, Yusupov N, & Toshpulatova S (2019). Soil salinization and its impact on cotton productivity in Uzbekistan. *Uzbek Journal of Agricultural Sciences* .3(1): 14–20.
18. Khamdullaev SA, Nabiev SM, Azimov AA, & Shavkiev JSH (2025a). Evaluation of drought tolerance in naturally colored cotton (*Gossypium hirsutum*) under water deficit conditions. *Regulatory Mechanisms in Biosystems*. 16(1): e25035.
19. Khamdullaev S, Nabiev S, Azimov A, Shavkiev J, & Yuldashov U (2021). Combining ability of yield and yield components in upland cotton (*Gossypium hirsutum* L.) genotypes under normal and water-deficit conditions. *Plant Cell Biotechnology and Molecular Biology*. 22(35&36): 176–186.
20. Khamdullaev ShA, Shavkiev JSh, & Azimov AA(2025b). The effect of abiotic stresses on plant productivity traits in Pima cotton genotypes. *European Journal of Agricultural Research*. 5. 74–79.
21. Kerstiens G (2006). Water transport in plant cuticles: An update. *Journal of Experimental Botany*, 57(11): 2493–2499.
22. Lawson T, & Blatt MR (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water-use efficiency. *Plant Physiology*. 164: 1556–1570.
23. Makamov A, Shavkiev J, Kholmuradova M, Boyqobilov U, Normamatov I, Norbekov J, Khusenov N, Kushakov SH, Yuldasheva Z, Khoshimov S, Buriev Z (2023). Cotton genotypes appraisal for morpho-physiological and yield contributing traits under optimal and deficit irrigated conditions. *SABRAO Journal of Breeding and Genetics*. 55(1): 74-89.
24. Makamov AKh, Kholmuradova MM, Khusenov NN, Boyqobilov UA, & Shavkiev JS(2022a). Assessment of drought tolerance in cotton genotypes. *Academic Research in Educational Sciences*. 3(6): 437–446.
25. Makamov AKh, Norbekov JK, Yuldasheva ZZ, Buriev ZT, & Shavkiev JS(2022b). Indicators of some morphological-economic traits of cotton genotypes under optimal and water-deficit conditions. *Academic Research in Educational Sciences*. 3(12): 65–75.
26. Matniyazova H, Nabiev S, Azimov A, Shavkiev J (2022). Genetic variability and inheritance of physiological and yield traits in upland cotton under diverse water regimes. *SABRAO Journal of Breeding and Genetics*. 54 (5): 976-992.
27. Monneveux P, Reynolds MP, & Trethowan R (2008). Drought adaptation in wheat. *Journal of Experimental Botany*. 59(11): 2767–2784.
28. Munns R, & Tester M (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*. 59:651–681.
29. Nabiev CM, Usmanov RM, Khamdullaev Sh A, Shavkiev J Sh (2020). Study of physiological indicators of the water balance of plants and morphological signs of leaf of fine-fiber varieties in different irrigation regimes. *Journal of biology of Uzbekistan*. 1:51-58.
30. Nabiev SM, Khamdullaev SA, Abdushukirova SK, & Shavkiev JS (2016). Study of economically valuable traits and their phenotypic correlations in medium-fiber cotton cultivars and lines under different water supply conditions. *Agricultural Sciences and the Agro-Industrial Complex at the Turn of the Century*. 82–86.
31. Narimonov A, Azimov A, Yakubjanova N, Shavkiev J (2023). Scientific basis of cotton seed germination in the Central Region of Uzbekistan. *SABRAO Journal of Breeding and Genetics*. 55(5): 1561-1572.
32. Netondo GW, Onyango JC, & Beck E (2004). Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science*. 44(3): 806–811.

33. Parida AK, & Das AB (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety*. 60(3): 324–349.
34. Roy SJ, Negrão S, & Tester M (2014). Salt resistant crop plants. *Current Opinion in Biotechnology*. 26: 115–124.
35. Samanov S, Arslanov D, Ernazarova Z, Iskandarov A, Muhammad X, Bunyod G, Gulomov G, Sirojjodinov B, Dusmatova G, & Shavkiev J (2024). The diversity and breeding potential of *G. hirsutum* L. genotypes based on the Uzbekistan cotton gene bank collection. *Journal of Wildlife and Biodiversity*. 8(4): 119–128.
36. Sanaev NN, Gurbanova NG, Azimov AA, Norberdiev TN, Shavkiev JS (2021). Inheritance of the “plant shape” trait of the varieties and introgressive lines of *G. hirsutum* L. in drought conditions. *Plant Cell Biotechnology and Molecular Biology*. 22 (25-26): 122-129.
37. Shavkiev J, Azimov A, Nabiev S, Khamdullaev S, Amanov B, Kholikova M, Matniyazova H, Yuldashov U (2021a). Comparative performance and genetic attributes of upland cotton genotypes for yield-related traits under optimal and deficit irrigation conditions. *SABRAO Journal of Breeding and Genetics*. 55(5): 53(2): 157-171.
38. Shavkiev J, Hamdullaev SH, Nabiev S, Usmanov R (2019). Water sensitivity and tolerance indices upon productivity in upland cotton and other economic valuable traits. *Bulletin of Gulistan State University*. 2:71-76.
39. Shavkiev J., Nabiev S., Azimov A., Chorshanbiev N., And Nurmetov K.H (2022). Pima cotton (*GOSSYPIMUM BARBADENSE* L.) lines assessment for drought tolerance in Uzbekistan. *SABRAO Journal of Breeding and Genetics*. 54 (3) 524-536.
40. Shavkiev Jaloliddin, Nabiev Saidgani, Abdulahat, Khamdullaev Shukhrat (2021b). Economic and physiological traits of Pima cotton lines in Uzbekistan and their correlation. *Universum: chemistry and biology*. 7 (85(2)): 14-22.
41. Shavkiev JS, Khamdullaev SA, Nabiev SM, Bozorov TA, Abdishukirova SK, & Eshonkulov ES. (2017). Pigment content in leaves of cotton cultivars under optimal water regime and drought. *The Way of Science*. 3: 16–18.
42. Shavkiev JS, Makamov AM, & Tulkinova KS. (2024). Some morphological characteristics of cotton genotypes with different origins under optimal and water-deficit conditions. *Modern biology and genetics*. 4(10): 56-67
43. Shavkiev J, Azimov A, Khamdullaev S, Karimov H, Abdurasulov F, & Nurmetov K (2023). Morpho-physiological and yield contributing traits of cotton varieties with different tolerance to water deficit. *Journal of Wildlife and Biodiversity*. 7(4): 214–228.
44. Shavkiev J, Azimov A, Khamdullaev S, Ahmedjanov A, Karimov E, Mamarasulov U, & Nurmetov X (2025). Multifactorial ANOVA analysis of genotype and environment effects on morphological and agronomic traits in *G. barbadense* L. cotton. *Journal of Wildlife and Biodiversity*. 9(2): 388–408. <https://doi.org/10.5281/zenodo.15521377>
45. Schonfeld MA, Johnson RC, Carver BF, & Mornhinweg DW (1988). Water relations in winter wheat: Excised-leaf water loss and drought resistance. *Crop Science*. 28(3): 526–531.
46. Slavík B (1974). *Methods of Studying Plant Water Relations*. Springer.
47. Szabados L, & Saviouré A (2010). Proline: A multifunctional amino acid. *Trends in Plant Science*. 15(2): 89–97.
48. Turner NC (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil*. 58(1–3): 339–366.

49. Tulkinova KS, Shavkiev JS, Azimov AA (2024). Study of drought tolerance indices of cotton genotypes. *Modern biology and genetics*. 4(10): 67-75.
50. Uddling J, Gelang-Alfredsson J, Piikki K, & Pleijel H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 readings. *Photosynthesis Research*. 91: 37–46.
51. Weatherley PE (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytologist*. 49: 81–97.
52. Yuldoshev A, Tursunov A, & Mamajonov A (2020). The importance of physiological and genetic criteria in creating salinity-tolerant cotton varieties. *Scientific Journal of Agriculture of Uzbekistan*. 2(5): 42–47.