



“MOLECULAR AND PHENOTYPIC EVALUATION OF COTTON (*GOSSYPIUM HIRSUTUM* L.) GENOTYPES FOR SALT TOLERANCE USING DNA MARKERS”

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ABSTRACT

*Soil salinity is a major constraint to cotton production in arid and semi-arid regions. This study evaluated 25 cotton (*Gossypium hirsutum* L.) genotypes using a combined molecular and phenotypic approach to identify salt-tolerant lines. Genomic DNA was extracted by the CTAB method and analyzed with SSR, SNP, and RAPD markers, while plants were tested under controlled NaCl stress (100–150 mM). The results revealed significant marker–trait associations: several SSR and SNP loci (including GhSNP12, GhSNP34, and GhSNP41) were strongly correlated with relative water content (RWC), biomass, and yield stability. Phenotypic evaluation showed that five lines (COT-17, COT-21, COT-25, COT-31, and COT-34) consistently outperformed controls, reducing yield losses to 12–15%, maintaining higher RWC (73–76%) and achieving superior stress tolerance indices ($STI = 0.82–0.88$). This integrated molecular and physiological analysis demonstrates that DNA marker-assisted selection is an effective strategy for identifying and developing salt-tolerant cotton genotypes. The identified lines provide valuable germplasm for breeding programs aimed at enhancing cotton resilience in saline-prone environments*

Introduction

Abiotic stresses, particularly soil salinity, are becoming increasingly destructive under the conditions of global climate change. Unlike drought or heat, salinity affects plants in a dual way: it causes both osmotic stress and ion toxicity, leading to impaired water uptake, nutrient imbalance, and growth suppression. Cotton (*Gossypium hirsutum* L.), although moderately tolerant to salinity compared to other major crops, still suffers severe productivity losses under high salt concentrations. Reports indicate that yield decline in sensitive cultivars may

reach 30–40%, which directly threatens the sustainability of cotton-based economies in arid regions. In recent decades, molecular genetics has provided new opportunities for accelerating crop improvement. Genome-wide tools such as SSR (Simple Sequence Repeats) and SNP (Single Nucleotide Polymorphisms) markers allow breeders not only to detect polymorphisms but also to link them to functional traits associated with stress adaptation. These approaches are central to marker-assisted selection (MAS), which enables rapid screening of large germplasm collections and precise identification of elite parental lines. Importantly, such strategies have already shown success in rice, wheat, and barley, but cotton research in this direction remains relatively underexplored. This study focuses on the genomic and phenotypic evaluation of 25 cotton genotypes using a combination of SSR, SNP, and RAPD markers. By correlating molecular variation with key physiological parameters such as relative water content (RWC), biomass accumulation, and stress tolerance indices, it aims to discover novel allelic combinations that underpin salinity tolerance. Unlike previous investigations that were limited to small sets of accessions or a narrow range of markers, the present work provides a comprehensive characterization of diverse germplasm under controlled NaCl stress conditions. The ultimate objective is to establish a set of DNA markers reliably associated with salt tolerance, which can serve as a foundation for modern cotton breeding programs. The integration of molecular marker data with field performance will create a pathway toward developing resilient cotton lines, ensuring agricultural stability in saline-prone environments and contributing to global food and fiber security.

Materials and Methods

This study was carried out on a set of 25 cotton (*Gossypium hirsutum* L.) genotypes originating from both national and international germplasm collections, with the objective of assessing their salt tolerance through a combination of molecular and phenotypic approaches. Control varieties commonly cultivated in the saline-affected regions of Uzbekistan (Bukhara and Karakalpakstan) were included to provide reference benchmarks. Genomic DNA was extracted from young leaf tissues using a modified CTAB method (Doyle & Doyle, 1990), which has proven effective for cotton tissues with high polysaccharide content. The quality and concentration of DNA were checked with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Three types of molecular markers were employed to capture genetic variation: 40 SSR primer pairs specific to cotton genome regions, 25 SNP loci previously associated with abiotic stress tolerance, and 15 RAPD primers for assessing polymorphism. Amplification was performed in a Bio-Rad T100 thermal cycler under standard PCR conditions, and products were separated on 2% agarose gels and visualized with a Gel Doc XR+ imaging system. In parallel with molecular screening, genotypes were subjected to controlled salinity stress experiments using NaCl concentrations of 100–150 mM, following protocols adapted from ICBA (2018) and USDA-ARS (2019). Both pot experiments and field trials were established to ensure reliable assessment. Phenotypic evaluation included the measurement of Relative Water Content (RWC) in leaves, basic biometric traits such as plant height, number of sympodial branches and raw fiber yield, as well as stress indices (Stress Tolerance Index – STI, and Tolerance index – TOL). These indicators were chosen based on their established relevance in earlier cotton and cereal studies (Ashraf & Foolad, 2007; Munns & Tester, 2008; Flowers & Colmer, 2015).

The integration of molecular and phenotypic datasets allowed us to establish robust marker–trait associations. Statistical analyses were performed using RStudio v.4.2.1 and SPSS v.26. Analysis of variance (ANOVA) was applied to test significance among genotypes, correlation analysis was conducted to detect associations between molecular markers and phenotypic traits, while Principal Component Analysis (PCA) and UPGMA clustering were used to classify genotypes into tolerant, moderately tolerant, and sensitive groups. Methodologically, this comprehensive design follows the framework of association mapping approaches successfully applied in cotton (Abdurakhmonov et al., 2010; Yang et al., 2014; Fang et al., 2017). Thus, the methodology ensured not only the identification of DNA markers linked to salinity tolerance, but also the simultaneous verification of their phenotypic relevance. This integrated approach strengthens the validity of the results and creates a solid basis for the marker-assisted development of salt-tolerant cotton lines that can be used in breeding programs for arid and saline environments.

Results

The evaluation of 25 cotton (*Gossypium hirsutum* L.) genotypes under NaCl-induced salinity stress (100–150 mM) revealed significant differences in physiological and yield-related traits. Table 1 presents a summary of the most promising newly developed lines compared to the control.

Table 1. Performance of cotton genotypes under salinity stress

Genotype	Yield Loss (%)	RWC (%)	Biomass (% of control)	STI
Control	32.5	58.3	100	0.55
COT-17	14.0	74.5	118	0.83
COT-21	13.5	75.0	120	0.85
COT-25	12.8	74.2	122	0.88
COT-31	15.0	73.8	119	0.82
COT-34	12.5	76.0	121	0.87

Integrated analysis and discussion of results

The data clearly show that the control genotype experienced the highest yield loss (32.5%) and the lowest relative water content (RWC = 58.3%), indicating severe susceptibility to salinity. In contrast, the selected lines (COT-17, COT-21, COT-25, COT-31, COT-34) demonstrated substantially reduced yield losses (12.5–15.0%) and consistently higher RWC values (73.8–76.0%). Biomass accumulation also supports this differentiation: while the control remained at baseline (100%), the tolerant lines exhibited a significant increase, ranging from 118% to 122% of the control. The stress tolerance index (STI) further confirms these findings: tolerant lines maintained values between 0.82–0.88, compared to only 0.55 in the control. Among the new lines, COT-25 showed the best overall performance, combining the lowest yield loss (12.8%), the highest biomass (122% of control), and the strongest STI (0.88). Lines COT-21 and COT-34 also performed exceptionally well, with balanced physiological and agronomic responses. These results align with earlier reports (Ashraf & Foolad, 2007; Yang et al., 2014; Fang et al., 2017) where high RWC and biomass stability were identified as key physiological markers of salinity tolerance. The strong

correlation between molecular markers (GhSNP12, GhSNP34, GhSNP41) and these traits further validates the marker-assisted selection (MAS) approach applied in this study.

Conclusion

The evaluation of 25 cotton genotypes under salinity stress identified five new lines (COT-17, COT-21, COT-25, COT-31, and COT-34) with significantly reduced yield losses (12–15%) and higher physiological stability compared to the control. These lines demonstrated improved RWC, greater biomass accumulation, and high STI values (0.82–0.88), confirming their potential as valuable sources for salt-tolerant cotton breeding.

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