Postprint: Identification and Evaluation of Drought Resistance at the Flowering and Boll-Forming Stage in an Upland-Sea Island Cotton Recombinant Inbred Line Population

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Abstract

Using an F2:6 recombinant inbred line (RIL) population derived from upland cotton material (Zhong 07) and sea-island cotton material (Xinhai 20) as experimental materials, field drought stress treatments were applied to the cotton RIL population during the flowering and boll-setting stage at Shihezi 144th Regiment in July of 2018 and 2019. Through measurement of agronomic traits and yield-related traits, and by combining analysis of variance, principal component analysis, and cluster analysis, drought resistance identification and evaluation of the RIL population were conducted. Principal component analysis revealed that boll number, lint yield, effective fruit branch number, and lint percentage exhibited more pronounced changes in the RIL population under drought stress conditions. Based on cluster analysis using drought resistance metric values (D values), the RIL population materials were divided into four groups: Group I was the drought-resistant type, comprising 16 lines including HL-44, HL-48, and HL-13; Group II was the moderately drought-resistant type, comprising 19 lines including HL-21, HL-8, and HL-19; Group III was the drought-sensitive type, comprising 29 lines including HL-11, HL-12, and HL-3; and Group IV was the highly drought-sensitive type, comprising 8 lines including HL-10, HL-18, and HL-31. The parental material Zhong 07 was identified as drought-resistant, while Xinhai 20 was identified as drought-sensitive. Additionally, five lines in the population exhibited stronger drought resistance than the parent Zhong 07, belonging to the drought-resistant group; and eight lines showed higher drought sensitivity than the parent Xinhai 20, belonging to the highly sensitive group. This indicates that the RIL population not only possesses the genetic characteristics of the parental upland and sea-island cotton in terms of drought resistance, but also exhibits transgressive genetic characteristics. This study provides a

foundation for future research on drought resistance in sea-land recombinant inbred line populations.

Full Text

Identification and Evaluation of Drought Resistance in Upland-Island Recombination Inbred Line Population at Blossoming and Boll-Forming Stages

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Abstract

The F2:6 upland-island recombination inbred line (RIL) population derived from drought-resistant upland cotton (Zhong 07) and drought-sensitive island cotton (Xinhai 20) was used as experimental material. Field drought stress experiments were conducted during the blossoming and boll-forming stages in Shihezi in July 2018 and 2019. Through measurement of agronomic and yield-related traits, combined with variance analysis, principal component analysis, and cluster analysis, the drought resistance of the upland-island RIL population was identified and evaluated. Principal component analysis revealed that boll number, lint yield, effective fruit branch number, and lint percentage showed the most significant changes under drought stress. Based on clustering by drought resistance comprehensive evaluation value (D value), the population was divided into four groups: Group I (drought-resistant) included 16 lines such as HL-44, HL-48, and HL-13; Group II (moderately drought-resistant) comprised 19 lines including HL-21, HL-8, and HL-19; Group III (drought-sensitive) contained 29 lines such as HL-11, HL-12, and HL-3; and Group IV (extremely drought-sensitive) included 8 lines such as HL-10, HL-18, and HL-31. The parental material Zhong 07 was identified as drought-resistant, while Xinhai 20 was drought-sensitive. Notably, five lines in Group I showed stronger drought resistance than Zhong 07, and eight lines in Group IV were more drought-sensitive than Xinhai 20. These results demonstrate that the upland-island RIL population not only inherited the genetic characteristics of both parental species but also exhibited transgressive segregation for drought resistance, providing a foundation for future research on drought resistance in such populations.

Keywords: cotton; upland-island RIL population; blossoming and boll-forming stage; drought stress; drought resistance evaluation

Introduction

Cotton is a crucial oil and economic crop, as well as a major source of textile fiber worldwide. In recent years, cotton cultivation in northwestern China has expanded substantially, with Xinjiang becoming the country' s largest cotton-producing region. However, cotton production is frequently affected by abiotic stresses, particularly drought, which inhibits growth and development, reduces yield, and can even cause plant death. Since cotton drought resistance is controlled by multiple genes and involves complex mechanisms, breeding for drought resistance is challenging, and suitable drought-resistant varieties are lacking in major cotton cultivation areas. Therefore, investigating drought resistance mechanisms and identifying stress-tolerant materials is essential.

The blossoming and boll-forming stage is the most critical period for reproductive development and water-fertilizer demand in cotton, with extremely high sensitivity to water conditions. Water deficiency during this stage significantly impacts both yield and fiber quality. Studies on upland cotton have shown that brief drought stress during the blossoming and boll-forming stage reduces effective boll number per plant, accelerates growth processes, shortens the boll period, increases shedding rate of buds and bolls in the lower and middle canopy, advances boll opening, reduces seed cotton and lint yield, but increases lint percentage. Consequently, water deficit during this period directly affects final yield and quality. Comprehensive drought resistance evaluation of island cotton germplasm resources has identified strong drought-resistant accessions and selected individual plant yield, boll weight, and plant height as suitable evaluation indicators.

The upland-island recombination inbred line population combines the excellent yield traits and broad adaptability of upland cotton with the superior fiber quality of island cotton. While previous research on such populations has focused primarily on heterosis, systematic evaluation of drought resistance remains limited. This study employed field drought stress during the blossoming and boll-forming stage to investigate agronomic and yield traits, using variance analysis, correlation analysis, cluster analysis, and comprehensive drought resistance evaluation to identify and screen drought-resistant materials in a population derived from drought-resistant upland cotton Zhong 07 and drought-sensitive island cotton Xinhai 20, providing a theoretical basis for cotton drought resistance identification and breeding.

Materials and Methods

Plant Materials

The experimental material consisted of an F2:6 upland-island recombination inbred line population provided by the Genetics and Breeding Laboratory of Xinjiang Agricultural University. The population was developed using drought-resistant upland cotton Zhong 07 as the female parent and drought-sensitive island cotton Xinhai 20 as the male parent, comprising 72 lines total. Zhong 07 is characterized by compact plant architecture, hairy stems, lodging resistance, medium leaf size, and smooth boll opening, while Xinhai 20 is an early-maturing, zero-type branching variety with a cylindrical plant shape and long oval bolls.

Experimental Design

Field experiments were conducted in 2018 and 2019 at the cotton breeding experimental field of Xinjiang Agricultural University in Shihezi (144th Regiment). Before sowing, two treatment areas were established: normal irrigation (control) and drought stress. Each cotton line was planted in a two-row plot with 10 cm plant spacing and 10 plants per row, separated by protective rows. Both treatments were replicated twice. A drip irrigation system under plastic mulch was employed with the configuration of "one drip line for two rows" in a (20-25 cm)+(50-60 cm) wide-narrow row pattern. Drought stress was imposed during the blossoming and boll-forming stage (around July 20) by withholding water for two cycles (7-10 days each), while the control received normal irrigation. After the stress period, normal irrigation resumed.

Trait Measurement

Agronomic traits including plant height, fruit branch number, effective fruit branch number, boll number, and effective boll number were measured from five uniform plants per line at the boll opening stage (around September 10). At harvest (around October 10), 50 bolls were collected from each line, weighed for seed cotton yield, then ginned to separate lint and calculate lint percentage (lint weight/seed cotton weight) and single boll weight.

Data Analysis

Data were compiled and calculated using Excel 2010. Statistical analysis was performed with SPSS 20.0 software. Drought resistance coefficient (DC) was calculated as DC = Xi/CKi, where Xi and CKi represent trait values under drought stress and control conditions, respectively. Data were standardized to eliminate genetic background effects. The subordinate function value, factor weight coefficient (ω), comprehensive drought resistance evaluation value (D), and broad-sense heritability (H^2) were calculated using the following formulas:

1. Subordinate function value: (Xi) = (Xi - Xmin)/(Xmax - Xmin)
2. Factor weight coefficient: ωi = Pi/ Pi, where Pi is the contribution rate of the ith principal component
3. Comprehensive evaluation value: D = Σ[(Xi) × ωi]
4. Broad-sense heritability: $H^2 = \sigma^2G/(\sigma^2G + \sigma^2E/nenr)$, where σ^2G is genotype variance, σ^2E is environmental variance, and nenr is genotype-environment interaction error.

Results

Phenotypic Traits of Parents and Population Under Different Water Conditions

Analysis of field phenotypic traits revealed that under different water conditions in both years, most traits in the population showed transgressive segregation, with maximum values exceeding parental maxima and minimum values below parental minima, except for seed cotton yield and single boll weight in 2018 under normal conditions [TABLE:1, TABLE:2]. Paired sample t-tests showed significant or highly significant differences between treatments for all traits in both years. In 2018, plant height, fruit branch number, effective fruit branch number, boll number, and effective boll number showed highly significant differences, while lint yield, lint percentage, single boll weight, and seed cotton yield showed significant differences. In 2019, plant height, fruit branch number, and effective fruit branch number were highly significantly different, while boll number, effective boll number, single boll weight, seed cotton yield, lint yield, and lint percentage showed significant differences. These results confirm that the drought stress treatment was effective and representative.

Variance and Generalized Heritability Analysis

Variance analysis across both years indicated that under different materials and water treatments, plant height, fruit branch number, effective fruit branch number, boll number, effective boll number, seed cotton yield, lint yield, lint percentage, and single boll weight were all significantly or highly significantly affected by genotype and water treatment, demonstrating substantial effects of both factors. Broad-sense heritability (H^2) ranged from 0.47 to 0.93 in 2018, with lint percentage showing the highest heritability and fruit branch number the lowest. In 2019, H^2 ranged from 0.53 to 0.92, with lint yield showing the highest heritability and plant height the lowest .

Correlation Analysis of Drought Resistance Coefficients

Correlation analysis of drought resistance coefficients (DC) revealed coordinated responses among traits . In 2018, highly significant positive correlations were observed between fruit branch number, effective fruit branch number, boll number, and plant height; between effective fruit branch number, boll number, effective boll number and fruit branch number; between boll number and effective boll number; and among lint yield, lint percentage, single boll weight, and seed cotton yield. Significant negative correlations were found between plant height and seed cotton yield, fruit branch number and seed cotton yield, effective fruit branch number and seed cotton yield, and boll number and seed cotton yield. In 2019, similar correlation patterns were observed, with effective boll number also showing significant positive correlation with plant height. These stable, strong correlations indicate consistent synergistic responses to drought stress among these trait groups across years.

Principal Component Analysis

Principal component analysis of DC values extracted key indicators for drought resistance evaluation . In 2018, two components were identified: boll number and lint yield, with a cumulative contribution rate of 70.47%. In 2019, three components were extracted: effective fruit branch number, lint yield, and lint percentage, with a cumulative contribution rate of 82.16%. The analysis revealed that boll number, lint yield, effective fruit branch number, and lint percentage were the most responsive traits to drought stress in this population.

Comprehensive Drought Resistance Evaluation

The D value, representing comprehensive drought resistance under stress conditions, was calculated by combining subordinate function values with factor weights from principal component analysis. D values ranged from 0.19 to 0.68 across the population, with a mean of 0.41 . Higher D values indicate stronger drought resistance. The top five lines were HL-44, HL-48, HL-13, HL-23, and HL-42, while the bottom five were HL-10, HL-18, HL-31, HL-11, and HL-12.

Cluster Analysis and Drought Resistance Classification

Systematic cluster analysis based on D values divided the population into four distinct groups [Figure 1: see original paper]. Group I (drought-resistant) contained 16 lines including HL-44, HL-48, and HL-13. Group II (moderately drought-resistant) comprised 19 lines such as HL-21, HL-8, and HL-19. Group III (drought-sensitive) included 29 lines like HL-11, HL-12, and HL-3. Group IV (extremely drought-sensitive) contained 8 lines including HL-10, HL-18, and HL-31. The parental materials were classified with Zhong 07 in the drought-resistant group and Xinhai 20 in the drought-sensitive group. Notably, five lines in Group I exhibited superior drought resistance compared to Zhong 07, while eight lines in Group IV were more drought-sensitive than Xinhai 20, confirming transgressive segregation for drought resistance in this population.

Discussion

Drought stress is among the most common environmental constraints affecting plant growth and development. Crop drought resistance is a complex quantitative trait controlled by multiple genes. Previous studies have demonstrated substantial phenotypic differences between drought-resistant and drought-sensitive genotypes under water-limited conditions. Cotton growth comprises several stages (seedling, budding, blossoming and boll-forming, and boll opening) with varying water requirements and drought response mechanisms. The blossoming and boll-forming stage is particularly critical, as water deficit during this period severely impacts yield and fiber quality.

Various comprehensive evaluation systems have been developed for different crops, with the D-value method widely recognized as reliable and objective. This approach considers both the importance of individual traits and their interrelationships, providing robust evaluation results. Studies in barley and cotton have confirmed that D-value-based clustering closely matches field performance and shows high heritability. Our study adopted this methodology to evaluate the upland-island RIL population, avoiding reliance on single-year data and providing more accurate assessment through two-year experiments.

Previous cotton drought research has focused primarily on upland cotton, with limited reports on island cotton. Our results identified Zhong 07 as drought-resistant and Xinhai 20 as drought-sensitive, consistent with earlier findings. The population exhibited transgressive segregation, with five lines surpassing the drought resistance of Zhong 07 and eight lines more sensitive than Xinhai 20. This demonstrates that the upland-island RIL population possesses not only the genetic characteristics of both parents but also novel variation beyond parental performance, providing valuable material for future drought resistance research.

Conclusion

This study employed field drought stress during the blossoming and boll-forming stage to evaluate an upland-island RIL population derived from Zhong 07 and Xinhai 20. Using variance analysis, heritability analysis, principal component analysis, and D-value evaluation, the population was classified into four groups: 16 drought-resistant lines, 19 moderately drought-resistant lines, 29 drought-sensitive lines, and 8 extremely drought-sensitive lines. The parental materials Zhong 07 and Xinhai 20 were confirmed as drought-resistant and drought-sensitive, respectively. Importantly, the population contained 5 lines with superior drought resistance to Zhong 07 and 8 lines more sensitive than Xinhai 20, indicating transgressive segregation. These results provide a foundation for future studies on drought resistance in upland-island recombination inbred line populations.

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Note: Figure translations are in progress. See original paper for figures.