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Marker Assisted Selection for Relative Water Content, Excised leaf Water Loss and Cell Membrane Stability in Cotton

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Abstract

Background: Drought stress is a major limitation in agricultural productivity. In cotton, drought tolerance is a multi-genic trait. The quantitative trait loci (QTLs), conferring drought tolerance in cotton, could be exploited for stress breeding using marker assisted selection.

Methods: We have screened drought related varieties of Pakistan using DNA markers to identify reported QTLs for drought tolerance. A total of 44 of these varieties were selected. All varieties were sown in the field to record relative water content, excised leaf water loss and cell membrane stability under drought stress condition. QTLs for relative water content, excised leaf water loss and cell membrane stability were checked from all varieties by using DNA markers NAU-2954, NAU-2715, NAU-6672, NAU-8406 and NAU-6790.

Results: Genotypic and phenotypic results showed that the QTL for relative water content *qtl*RWC-1 present on chromosome 23, linked marker NAU-2954, could be a major QTL conferring drought tolerance in cotton. Using Marker Assisted Selection the variety CRIS-134 showed all concerned QTLs for drought tolerance.

Conclusion: QTL for relative water content *qtl*RWC-1 could be a major QTL for drought stress tolerance in cotton. The variety CRIS-134 may be used for breeding drought tolerant cultivar.

Introduction

For agricultural crops, availability of fresh water is declining day by day. Water contents in plants are decreased as soil moisture level decreases [1]. As stomatal conductance became low, CO_2 uptake decreased [2]. Under drought stress, ATP content decreases as the drought is increased [3]. Under drought stress, normal functioning in plants is disturbed [4]. As compared with other crops, drought stress drastically affects cotton, the most important fiber crop of the world [5-9]. In cotton, drought stress lowers leaf water content and cell membrane stability [10-12], cellular growth [13,14], roots and stem growth [15], number of bolls per plant [16] and ultimately reduces yield [17,18].

Drought stress tolerance in cotton is a complex quantitative trait. The traits contributing drought tolerance are morphological as well as physiological such as relative water content, excised leaf water loss and cell membrane stability. The mechanism in plant which helps maintain water content in leaves during drought stress could enhance drought tolerance [19,20]. The most important defense trait under drought stress is relative water content, used as measurement of water retention capacity in plants [21,22]. Similarly, another defense mechanism under drought stress is to maintain cell membrane integrity [23]. The exploitation of genetic control of these traits could help enhance breeding for drought tolerance.

In crop plants, QTLs mapping for many important traits, such as yield, quality, disease resistance and drought tolerance is an ongoing research objective for researchers around the world. The identification of genomic regions carrying genes associated with quantitative trait called quantitative trait loci. DNA markers linked to the QTLs could be used for selection or screening of germplasm to find QTLs for the traits of interest. In cotton, QTLs mapping has been conducted for various traits such as fiber traits [24,25], boll traits [26], chlorophyll contents [27-29], earliness [30-32] and yield traits [33-36].

QTLs for the traits conferring drought tolerance such as relative water content [37,38], excised leaf water loss [38], cell membrane stability [37], osmotic potential and osmotic adjustment [39] has been identified. DNA markers linked with these traits could be used for marker assisted selection for drought tolerance in cotton.

Using DNA markers for various important traits, selection/screening/validation has been conducted for many traits such as disease resistance in tomato [40], fragrance genes in rice [41] and in wheat for rust resistance [42]. Very little attention has been given to screen germplasm for drought tolerance using DNA markers along with phenotypic screening. This research work aims to use marker assisted selection to screen out variety/genotype containing QTLs for drought tolerance.

Methods

All 44 varieties (Table 2) were selected on the basis of relevancy with drought tolerance. The collected varieties were evaluated using RCBD in three replications. In each replication, there were two rows for each variety. Each row comprised of ten plants, planted at 30cm plant to plant distance and 75 cm row to row distance. Irrigation was applied once after 40 days of planting. Physiological data related to drought tolerance was recorded when plants, under drought stress, showed effects of drought stress.

Relative Water Content (RWC)

Leave samples from plant were taken for analysis. After fresh weight, all samples were dipped in water to measure turgid weight. Finally the samples were oven dried (70°C) to measure dry weight. The RWC was calculated using formula as by [43].

$$RWC = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100$$

DNA Markers	Chr. No	QTLs	Ref.	QTL for the Trait
NAU-2954	23	*qtl*RWC-1	[38]	Relative Water Content
NAU-2715	12	*qtl*RWC-2	[38]	
NAU-6672	A5	*qtl*RWC1	[37]	
NAU-8406	A7	*qtl*ELWL	[37]	Excised Leaf Water Loss
NAU-2954	23	*qtl*ELWL-1	[38]	
NAU-6790	A1	*qtl*CMS	[37]	Cell Membrane Stability

Table 1: DNA markers used in the study, Chromosome no, QTLs name and Trait related to drought tolerance in cotton

Excised Leaf Water Loss (ELWL)

Three leaves per plant were taken. Using electric balance, fresh weight was measured, followed by keeping leaf samples on bench at normal room temperature. Wilted weight was recorded after twenty four hours. Finally samples were oven dried (at 70°C) to record dry weight. Using formula given by [44] ELWL was calculated

$$\text{ELWL} = [(\text{Fresh weight-Wilted weight})/\text{Dry weight}]$$

Cell Membrane Stability (CMS)

From each plant, three leave samples were used to measure CMS using following formula by [45]:

$$\text{CMS}\% = \frac{1-(\text{T1}/\text{T2})}{1-(\text{C1}/\text{C2})} \times 100$$

Where, T1= Stress sample conductance before autoclaving.
T2= Stress sample conductance after autoclaving
C1= Control sample conductance before autoclaving
C2= Control sample conductance after autoclaving

DNA Marker Studies

The leaves of all 44 varieties were used for DNA extraction. The selected leaves were frozen at -80°C. DNA was extracted by using standard CTAB method [46]. The DNA samples were checked for quality by gel electrophoresis. The DNA samples giving smear in the gel were rejected and only good quality DNA was selected for PCR studies. All 44 varieties were screened with DNA markers (Table 1). These markers were selected for relative water content, excised leaf water loss and cell membrane stability. PCR products were run on 1% agarose gel. Results were saved through Gel Documentation.

Results

The analysis of variance showed that all 44 varieties showed significant variation for the traits relative water content, excised leaf water loss and cell membrane stability (Table 2 in supplementary data). The data for physiological traits is given in Table 1. DNA marker studies shows that among 44 selected drought tolerant varieties, few varieties showed containing QTLs for drought tolerance. DNA marker 2954, band size 150bp, linked with *qtl*RWC-1 and *qtl*ELWL at chromosome no 23 [38] was appeared in variety 124-F, BH-36, CIM-448, NIAB-999, CIM-707, 149-F, MNH-93, CRIS-134, 199-F, CIM-506, MS-39, CIM-482, LSS, NIAB-78, MNH-552, CIM-109, BH-118, B-557, 268-F, MNH-554, 238-F, 4F and CIM-70 (Fig. 1) depicts presence of both QTLs. The size of band was similar as reported for these QTLs [38]. Phenotypic data (Table 2) also shows high relative water content with an average of 70% and low excised leaf water loss with an average of 2.1 in these genotypes, showing tolerance against drought stress.

DNA marker NAU-2715 linked with *qtl*RWC-2 [38] was present in NIAB-111, AC-134, MNH-147, S-12, CIM-707, BH-160, 149-F, MNH-93, CRIS-134, 199-F, S-14, MS-39, CRIS-9, K-68/9, MNH-129, B-557, MS-40, 216-F and 216-F (Fig. 1). Phenotypic data (Table 2) shows moderate water percentage with an average of 55%. DNA marker NAU-6672 linked with *qtl*RLWC1 [37] was appeared only in MNH-147, MNH-93 and CRIS-134. DNA marker NAU-8406 linked with *qtl*ELWL [37] was detected only in NIAB-111, MNH-552 and CRIS-134 with moderate values of excised leaf water loss (Table 2 in supplementary data). Genotypic and phenotypic data shows that this QTL may not be considered as major contributor for the trait.

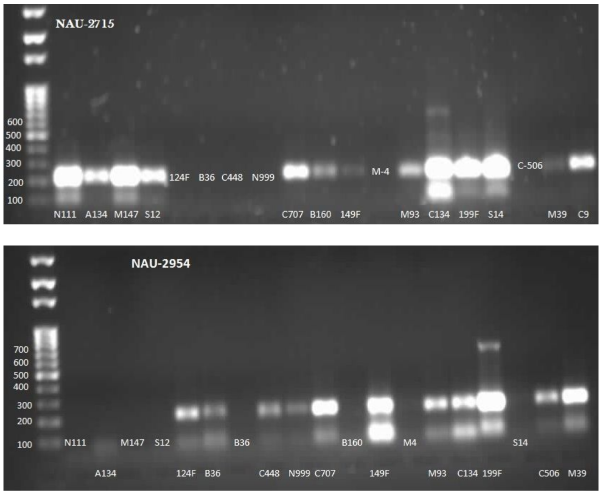

Figure 1: Screening of cotton varieties using DNA markers NAU-2954 and NAU-2715

Discussion

Screening with DNA markers and recording of phenotypic data shows that the DNA marker NAU-2954 linked with two QTLs, one for relative water content *qtl*RWC-1 and one for excised leaf water loss *qtl*ELWL contribute high drought tolerance in cotton. This QTL was reported to be mapped on chromosome no 23 at LOD=2.74 [38]. The additive effect for this QTL was 4.87, showing high inheritance. The varieties also showed high drought tolerance for relative water content and excised leaf water loss (Table 1). High drought tolerance was associated with presence of these QTLs shows that these QTLs may be considered as major contributors for drought tolerance in cotton. To breed drought tolerant cultivars, the genotypes carrying these QTLs may be included in the breeding programs.

Although a numbers QTLs has been detected for many important traits in many crops but less efforts has been made for examination of these QTLs in germplasm. In this research work some important QTLs has been screened in cotton varieties, considered as drought tolerant in Pakistan. Among these some varieties showed high relative water content as well as showed presence of QTL relevant to the trait. Similarly some varieties showed low excised leaf water loss as well as QTL for the trait. Among these, some varieties showed high relative water content and low excised leaf water loss as well as two QTLs namely *qtl*RWC-1 and *qtl*ELWL like CRIS-134. The data represents the high value of these QTLs for drought tolerance in cotton. A variety MNH-554 was tolerant in physiological screening but with absence of QTLs under study, depicts the need of further research for major QTLs related for drought tolerance.

The presence of DNA marker NAU-2954, linked with *qtl*RWC-1 and *qtl*ELWL on chromosome 23 shows high drought tolerance in cotton. The variety CRIS-134 carries QTLs for drought tolerance and may be selected for breeding programs. These QTLs showed high tolerance against drought stress in cotton.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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