MAPPING OF CANDIDATE GENES AND QUANTITATIVE TRAIT LOCI FOR DROUGHT TOLERANCE IN A RECOMBINANT INBRED LINE POPULATION OF GOSSYPIUM BARBADENSE

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Abstract

Drought is one of the major abiotic stresses that adversely affect cotton production. The objective of this study was to identify quantitative trait loci (QTLs) for drought tolerance in *Gossypium barbadense* cotton. Seedlings of 142 recombinant inbred lines (RILs) derived from the intraspecific cross of 'Dandara' × 'Giza 70' were evaluated in the greenhouse under 5% PEG treatment and control (water) conditions using a hydroponic system. Pant growth traits including plant height, fresh shoot weight and root weight, and physiological traits including chlorophyll content, evapotranspiration and leaf temperature, were measured. The experiment was a randomized complete block design with three replicates and repeated once. The parental line Dandara exhibited higher values for the traits than Giza 70 and significant genotypic variations were detected within the RIL population. Herritabilities for the traits were moderate to higher and were higher under the control conditions. The traits were also significantly and positively correlated except for the correlation between root weight and leaf temperature in the first test. Based on a linkage map comprised of 225 marker loci which were assembled into 32 linkage groups, five QTLs were detected including three for morphological traits and two for physiological traits, two of which were associated with drought responsive genes. These QTLs each explained 15.5 to 26.3% of the phenotypic variation. The results will be useful to enhance yield and its components in cotton under water stress conditions.

Introduction

Cotton is the leading fiber crop in the world. However, drought stress remains one of the major abiotic stresses lowering cotton yields in the U.S. and worldwide. Drought tolerance is a complex trait in cotton, which involves many genes and multiple metabolism pathways. So far, only a few drought responsive genes have been reported in cotton and more genes related to drought are needed to uncover the mechanism of drought tolerance in cotton (Zhang et al., 2009). Many traits have been suggested to be important to drought tolerance in cotton. Some of these are morphological ones including plant height, root characteristics, shoot growth rate, and root–shoot ratio (Cook, 1985; Malik, 1979); and others are physiological traits including leaf water content, chlorophyll content, and carbon isotope discrimination, and photosynthetic rate (Nepomuceno et al., 1998). All of these traits can be used as indicators of presence or absence of drought tolerance in cotton.

With the advent of molecular markers, great advances have been made in plant genome research. There are different types of molecular markers that can be used to enhance plant improvements. The first type is called phenotypic or morphological markers. In this type plant breeders make phenotypic selection based on the gene they are looking for and the association between this gene and other measured plant traits, and this type of markers follow Mendelian inheritance. The second set of markers is called biochemical markers including proteins and isozymes. This type of markers is less affected by environmental factors than morphological markers, and they are also codominant. The third set of markers is called DNA markers (Gupta et al., 2001) which are divided into three generations: the first generation- RAPD and RFLP, the second generation- AFLPs and SSRs, and the third generation- ESTs and SNPs. These markers can be used to improve cotton. However, most of these DNA markers are anonymous in the genome and some of them are associated with chromosomal regions determining quantitative trait loci (QTLs).

A more direct approach to study quantitative traits such as drought tolerance is through the use of candidate genes. The candidate gene approach consists of three steps. First, candidate genes are identified based on molecular and physiological studies or based on linkage data. Second, molecular polymorphism must be correlated with traits to identify the candidate genes on a genetic linkage map to determine the relationship between the candidate genes and the QTLs or to calculate statistical correlations between candidate genes and phenotypic variation. Third, the

candidate genes must be validated after identified from the first two steps (Pflieger et al., 2001). The objectives of this study were to, (1) evaluate drought tolerance in a recombinant inbred line (RIL) population (*Gossypium barbadense*) developed from a cross between a drought-tolerant Egyptian cultivar Dandara and a drought-sensitive cultivar Giza 70; (2) develop drought responsive gene markers; and (3) identify QTLs for drought tolerance anchored by molecular markers including drought responsive genes.

Materials and Methods

Plant materials

A RIL population of 142 lines (*G. barbadense*) was produced from a cross between Dandara and Giza 70. Dandara is drought tolerant with high yield, early maturity, and low fiber quality, while Giza-70 is drought sensitive with low yield, late maturity, and high fiber quality.

PEG treatment

The polyethylene glycol (PEG) treatment was conducted in the greenhouse at Fabian Garcia Research Center, New Mexico State University, Las Cruces, NM. PEG with a high molecular weight can mimic osmotic stress and is frequently used for drought tolerance studies in plants. In this study, the RILs and the two parents were grown in 2.5-in plastic pots and arranged in a randomized complete block design with 3 replicates for the PEG treatment and the same design was also used for the control. After the second true leaf emerged, seedlings for the PEG treatment were transferred to a hydroponic system containing 5% PEG solution, while seedlings for the control were transferred to another hydroponic system containing water. The irrigation system was run for 30 min every day for three weeks. The same experiments were repeated twice (Test 1 and 2).

Trait measurements

After three weeks of treatment, seedlings were measured for morphological traits including plant height (PH), fresh shoots weight (SW) and fresh root weight (RW), and for physiological traits including chlorophyll content (CC), leaf temperature (LT) and evapotranspiration (ET).

Data analysis

Analyses of variance (ANOVA) and correlation were performed using SAS 2000, and broad-sense heritabilities were estimated based on ANOVA.

DNA extraction

DNA was isolated from unfolded young leaves using the quick mini-prep method (Zhang and Stewart, 2000).

Genotyping

Out of the 142 RILs, 94 lines were randomly selected for genotyping. These 94 lines were evaluated for two different types of DNA markers including 250 SSR (simple sequence repeats), and 150 SSCP (single strand conformation polymorphism) designed based on candidate drought responsive genes.

Bulked sergeant analysis

Based on the screening in the greenhouse under the PEG conditions, 10 drought-tolerant and 10 drought-sensitive lines were selected among the 142 RILs tested. Equal amount of DNA of the selected lines were pooled into two separate bulks. SSR primers were then screened on the parents and two bulk DNA samples.

PCR reactions and gel electrophoresis

For SSR markers, the polymerase chain reactions followed Zhang et al. (2002). For SSCP analysis, the PCR reactions and gel electrophoresis followed Lu et al. (2009). Gel staining protocol was described by Benbonza et al. (2006).

Linkage mapping and QTL analysis

Join Map, version 4.0 software (Van Ooijen and Voomps, 2001) was used to perform a linkage analysis. The LOD threshold was 2.0. The Kosambi mapping function (Kosambi, 1944) was used to obtain genetic distances in centiMorgans (cM). The QTL analysis was conducted using QTLNetwork-2.0 (Yang et al., 2008) based on composite interval mapping and the Monte Carlo Chain (MCMC) was used to estimate QTL effects.

Results and Analysis

Analysis of variance, trait heritability and correlations

The combined analysis of variance showed significant genotypic variation for all morphological and physiological traits measured (Table 1 and 2). Significant variations in morphological and physiological traits were detected in the tests containing 142 RILs and the two parents- Danadar and Giza 70. Dandara exhibited higher value for all the traits especially under the PEG conditions.

Heritability estimates for all the traits measured under the PEG stress and control ranged from 55 to 85%. Significantly positive correlations were detected between all the traits except for the correlation between leaf temperature and root weight in the first test.

Table 1. Mean squares of combined analysis of variance over the first and second test for morphological traits.

Source of variation	df	Plant height	Fresh shoot weight	Fresh root weight
Test	1	4002.90**	1075.65**	24.00**
Genotype	143	64.33**	0.79**	0.33**
Treatment	1	3284.70**	750.37**	21.02**
Genotype x Test	143	21.50**	0.49**	0.25**
Test x Treatments	1	4482.90**	258.70**	0.70**
Genotype x Treatment	143	50.90**	0.75**	0.04**
Genotype x Treatment x Test	143	27.15**	0.17**	0.054**
Error	1152	5.34	0.03	0.02

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.

Table 2. Mean squares of combined analysis of variance over the first and second test for physiological traits.

Source of variation	df	Chlorophyll content	Evapotranspiration	Leaf temperature
Test	1	6052.90**	1020.32**	5002.25**
Genotype	143	74.32**	20.21**	65.21**
Treatment	1	4000.00**	900.00**	3210.52**
Genotype x Test	143	25.30**	15.80**	24.50**
Test x Treatments	1	5210.89**	190.87**	3214.51**
Genotype x Treatment	143	72.05**	5.45**	62.51
Genotype x Treatment x Test	143	33.25**	4.20**	28.75**
Error	1152	3.20	1.02	2.08

* Significant at 0.05 probability level. ** Significant at 0.01 probability level.

A genetic linkage map and QTL analysis

A total of 225 loci were assembled into 32 linkage group with 2-63 markers per group, which detected five main-effects QTLs under the control or PEG conditions. The information for the QTLs is summarized in Table 3 and Figure 1. Three QTLs were detected under the PEG conditions for plant height, root weight, and chlorophyll content and the superior alleles of the QTLs were all originated from the Dandara parent. Two QTLs were detected under the control conditions for shoot weight and evapotranspiration and the superior alleles of the two QTLs were from the other parent Giza 70. The results confirmed that Dandara was more drought tolerant than Giza 70. These QTLs explained 15.5- 26.3% of the phenotypic variation. All the QTLs were assigned to chromosomes according to Liu et al. (2000) and the cotton marker database (www.cottonmarker.org). The QTL for plant height under the PEG conditions was assigned to chromosome 2, the QTL for chlorophyll content was assigned to chromosome 9; the QTL for shoot weight was assigned to chromosome 22; the QTL for evapotranspiration was assigned to chromosome 25; and the QTL for root weight was assigned to chromosome 1. Interestingly, The QTL for root weight under the PEG conditions and the other for evapotranspiration under the control conditions were associated with candidate genes.

Table 3. QTLs detected by QTLNetwork2.0 for plant height (PH), shoot weight (SW), root weight (RW), chlorophyll content (CC), and evapotranspiration (ET) under the PEG and control conditions.

Trait	QTL name	Treatment	Marker interval	Chromosome	P-value	Superior parent	PV%
PH	7-2	PEG	BNL2847-250~BNL3442-300	2	0.0000	Dandara	15.5
SW	24.1	Control	BNL 3431-250~BNL4049-420	22	0.0006	Giza 70	23.1
RW	33-2	PEG	SNP 29-350~BNL 3590-380	1	0.0000	Dandara	17.6
CC	10-2	PEG	BNL 3537-280~BNL 3590-380	9	0.0000	Dandara	12.0
ET	42-1	Control	SNP 19-900~SNP 2-1000	25	0.0000	Giza 70	21.6

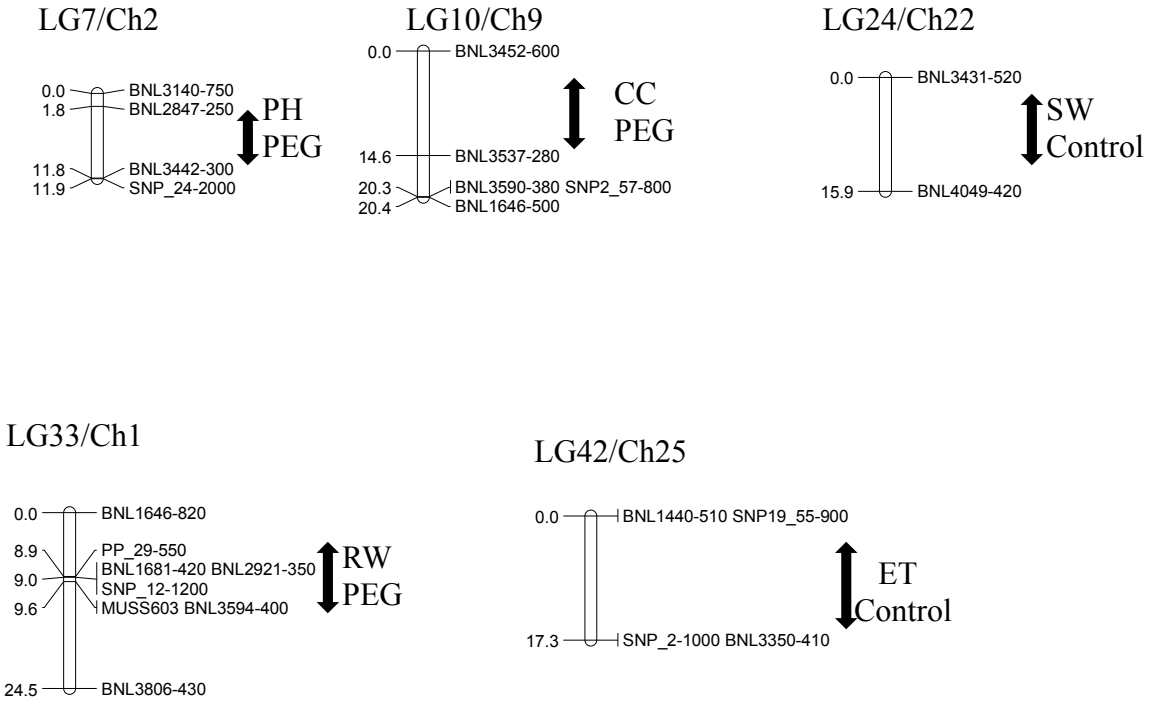

Figure 1. A linkage map of SSR and SSCP markers carrying QTLs for drought tolerance. The arrows identify the interval markers and the position of QTL for plant height (PH), fresh shoot weight (SW), root weight (RW), chlorophyll content (CC), and evapotranspiration (ET).

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