GENETIC ANALYSIS AND QTL MAPPING OF DROUGHT TOLERANCE IN COTTON UNDER PEG CONDITIONS

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Abstract

Drought is one of the major aboitic stresses that adversely affect cotton production. The main objective of this study was to identify quantitative trait loci (QTLs) for drought tolerance in cotton. Seedlings of 142 backcross inbred lines (BILs) derived from the interspecific cross of 'Sure-Grow 747' × 'Pima S-7' were evaluated for plant height, fresh shoot weight and root weight in the greenhouse under 5% PEG treatment and control (water) conditions using a hydroponic system. The experiment was a randomized complete block design with three replicates and repeated once. Selected BIL lines were also genotyped using molecular markers including AFLP, cDNA-AFLP, PAAP-AFLP, SSR and STS. The analysis of variance for the two tests was performed separately and combined. Significant variations due to genotype, treatment, test, genotype x test, genotype x treatment, and genotype x treatment x test were detected for all the traits except for genotype x treatment x test interaction for fresh shoot weight. Significant differences were detected within the BIL population for all the traits and Pima S-7 had taller seedlings with heavier shoot and root weight than Sure-Grow 747. Heritabilities for the three traits were moderate to high and were higher under control conditions. The three traits were also significantly and positively correlated between each other. Based on a linkage map comprised of 242 loci which were assembled into 41 linkage groups and covered 842 cM of the cotton genome, seven QTLs were detected including three for plant height, two for fresh shoot weight, and one for fresh root weight. These QTLs each explained 13.8 to 24.3% of the phenotypic variation and will be useful to enhance yield and its components in cotton under water stress conditions. More markers will be developed for a genome-wide scanning of QTLs for drought tolerance.

Introduction

Cotton is the most important fiber crop and the second most important oilseed crop in the United States. However, drought remains one of the major abiotic stresses lowering cotton yields in the United States and worldwide. Drought tolerance is a complex trait in cotton, which involves the coordination of many genes and multiple metabolism pathways with multigenic components (Leopold, 1990; Ingram and Bartels, 1996; Cushman and Bohnert, 2000). So far, only a few drought tolerance genes have been reported in cotton and breeders needs to know more genes related to drought to understand the mechanism of drought tolerance in cotton (Zhang et al., 2009).

Many parameters have been suggested as important relative to drought tolerance in cotton, and also several attempts have been made to combine physiological and morphological parameters to develop effective screening methods for drought tolerance. Morphological parameters include plant height, root characteristics, shoot growth rate and root-shoot ratio (Cook, 1985, Taylor, 1983, Ludlow and Muchow, 1990, Erssa et al., 1983, and Malik et al., 1979), and physiological traits include leaf water content, carbon isotope discrimination (Leidi et al., 1999), stomatal conductance, photosynthetic rate (Nepomuceno et al., 1998), and reduced transpiration (Quisenberry et al., 1982). These traits may be used as indicators of presence or absence of drought tolerance in cotton. Furthermore, many reports have used these traits to study the response of cotton genotypes to drought under greenhouse conditions (Loffroy et al., 1983; Ball et al., 1994), field conditions (Turner et al., 1986), or humid environments (Pettigrew, 2004).

Many approaches have been used to enhance drought tolerance in plants. One approach is called direct selection by screening germplasm under stress conditions and selecting the highest yielding genotypes. However, this phenotypic selection method is difficult to make progress for yield and its components under drought stress conditions as they are complex traits that are not only influenced by genotypes, but also by environmental factors and genotype by environmental interaction with low heritabilities under drought stress conditions (Smith et al., 1990; Ribaut et al., 1997; Rosielle and Hamblin, 1981). The second approach uses indirect selection for secondary traits. The effectiveness of selection for secondary traits to enhance yield under water stress conditions has been documented in cotton (Saranga et al., 1999; Wang et al., 2006; Seaad et al., 2011), maize (Chapman and Edmeades, 1999), wheat

(Ricards et al., 2000), and sorghum (Tuinstra et al., 1998). However, this method needs to estimate the correlation between secondary traits and yield and uses these traits with high correlations with yield. The third approach suggests testing of germplasm under stress and non-stress conditions and ranking genotypes for drought tolerance/susceptibility on reduction of the yield (Blum, 1988). Other yield-based estimates of drought tolerance include geometric mean (Fernandez, 1993), and drought susceptibility index (Fischer and Maurer, 1978). These approaches are categorized as conventional breeding, and they are time consuming and restricted by growing season. So far, breeding progress with these approaches is still limited. Another approach is biochemical method with polyethylene glycol (PEG). PEG with high molecular weight can mimic osmotic stress and therefore has been extensively used for drought tolerance studies in plants (James and Murray, 1979). In fact, PEG is considered one of the most effective solutions to evaluate and screening genotypes for drought tolerance.

With the advent of the molecular marker technology great advances have been made in plant genome research. Molecular markers are another approach that could be used in breeding for drought tolerance. Molecular markers have provided plant breeders with a rapid and powerful approach in selection, and give them more genetic information, which help them to determine the criteria of selection. Furthermore, a combination of molecular markers with conventional breeding methods can help to achieve the best results in selection. The current primary molecular breeding approaches include transgenic modification and quantitative trait loci (QTLs) with marker-assisted selection. One of the main applications of QTL mapping is using marker-assisted selection (MAS) as a tool to enhance plant breeding (Lande and Thompson, 1990), so breeders can use MAS as indirect selection for desirable genes. The first molecular map of cotton was reported by Renisch et al. (1994). This map was based on an interspecific F2 population, included 705 restriction fragment length polymorphism (RFLP) loci placed onto 41 linkage groups with a total length of 4675 cM. A number of other linkage maps have been reported since then (i.e., Shappley, 1996; Shappley et al., 1998; Ulloa and Meredith, 2000; Kohel et al., 2001; Ulloa et al., 2002; Zhang et al., 2002; Mei et al., 2004). However, only a few linkage maps with QTLs related to drought tolerance in cotton have been reported due to the lack of genetic information and difficulties in research for drought tolerance in cotton (Saranga at al., 2004; Levi et al., 2008; Babar et al., 2009; Saeed et al., 2011).

The objectives of this study were to, (i) evaluate the performance of a backcross inbred line (BIL) population under PEG treatment; and (ii) identify QTLs for drought tolerance.

Materials and Methods

Plant Materials
One hundred and forty two backcross inbred lines (BILs) derived from a cross between Pima S-7 (*Gossypium barbadense* L.) and Sure-Grow 747 (*G. hirsutum* L.) were used as the mapping population. Pima S-7 is a commercial cultivar with high fiber quality and tolerant to drought stress (Turcotte et al., 1992). Sure-Grow 747 is a commercial Upland cotton with high yield but sensitive to drought with low fiber quality.

PEG Stress Treatment
The PEG treatment was conducted in the greenhouse at Fabian Garcia Plant Science Center, New Mexico State University, Las Cruces, NM. 5% PEG 8000 (with molecular weight of 8000) solution was used as the treatment and water was used as the control. The 142 BILs and the two parents were grown in 2.5'' plastic pots and arranged in a randomized complete block design with 3 replicates (1 plant/entry/replicate) 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 in the same design were transferred to another hydroponic system containing tap water. The irrigation system was run for 30 min every day for three weeks. The same experiments were conducted twice (Test 1 and 2).

Trait Measurements
After three weeks of treatment, seedlings were measured for three traits including plant height (PH, cm), fresh shoot weight (SW, g/plant), and fresh root weight (RW, g/plant). For Test 2, leaf wilting and/or yellowing were noted in the PEG treatment (Figure 1 and 2). After three weeks of PEG treatment, all individual plants were also scored for drought tolerance using a drought tolerance rating system, as the following: 0- normal growth; 1- yellowing cotyledons; 2- wilting cotyledons; 3- wilting true leaves; 4- dry plant; and 5- dead plant.

Figure 1. Differences in seedling growth between the control (left) and PEG treatment (right).

Figure 2. Phenotypes of individual plants after PEG treatment. 0- normal growth; 1- yellowing cotyledons; 2-wilting cotyledons; 3-wilting true leaves; 4- dry plant; 5- dead plant.

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

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

Genotyping
Out of the 142 BILs, 93 lines were randomly selected for genotyping. These 93 lines were evaluated for different types of DNA markers, including 228 AFLP (amplified fragment polymorphism), 40 PAAP-AFLP (promoter anchored amplified polymorphism-AFLP), 141 cDNA-AFLP, 42 STS (sequence tagged sites), and 150 SSR (simple sequence repeat) markers (Adams, 2011). All these markers are PCR-based.

Linkage Map and QTL Analysis
Join Map, version 4.0 software (Van Ooijen and Voomps, 2001) was used to perform linkage analysis. The threshold LOD score was 2.0. The Kosambi mapping function (Kosambi, 1944) was used to obtain genetic distances in centiMorgans (cM). The QTL analysis was carried out using QTLNetwork-2.0 (Yang et al., 2008) using composite interval mapping and the Monte Carlo Chain (MCMC) was used to estimate QTL effects. The QTL names are assigned using the nomenclature system given at CottonDB.org using an A.B-C.D system, where A is the abbreviation given for the trait in CottonDB.org; B is the initials of the parents of the mapping population (SG 747 and Pima S-7); C is the linkage group name, and D is the QTL's order.

Results and Analysis

Analysis of Variance and Phenotypic Variation

Combined analysis of variance showed significant genotypic variation for all the traits, and test, treatment, interactions between genotype and test or treatment were all significant except for genotype x test x treatment for fresh shoot weight (Table 1). Significant differences in plant height, and fresh shoots and roots weights were detected between BILs and also between Pima S-7 and Sure-Grow 747 in both Test 1 and 2.

Table 1. Mean squares of combined analysis of variance over Test 1 and 2.

Source of variation	D.F.	Plant height	Fresh shoot weight	Fresh root weight
		cm	g/plant	g/plant
Test	1	5582.9**	1065.65**	22.99**
Genotype	143	74.33**	0.69**	0.30**
Treatment	1	50804.70**	850.37**	20.43**
Genotype x Test	143	31.15**	0.59**	0.30**
Test x Treatment	1	5582.90**	258.70**	0.69**
Genotype x Treatment	143	40.09**	0.65**	0.03**
Genotype x Treatment x Test	143	29.15**	0.57	0.05**
Error	1152	6.34	0.37	0.03

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

The PEG treated seedlings were much shorter than the control plants (Figure 1). The Pima cotton parent was taller with heavier shoot and root weight than the Upland cotton parent under both control and PEG conditions (Table 2). Furthermore, the relative reduction due to PEG treatment in the Pima parent for all the traits was lower than that in the Upland cotton parent. All traits followed normal distributions (skewness and kurtosis less than 1.0). Heritabilities for the three traits were moderate (0.559) to high (0.947) under both PEG and control conditions (Table 2). Interestingly, all of the heritabilities values under the PEG treatment were lower than these under the control conditions, consistent with earlier reports that heritabilities were decreased under drought stress.

Table 2. Average plant height (PH), fresh shoot weight (SW) and fresh root weight (RW) of parents, BILs, relative reduction (RR), skewness (sk), kurtoris (ku), and broad-sense heritabilities (Hb).

Trait	Test	Treatment	Pima S-7	RR (%)	SG 747	RR (%)	BILs Min	BILs Max	BILs Mean	BILs RR	Sk	Ku	Hb (%)
PH (cm)	1	PEG	25.8	21.8	15.0	40.0	8.6	24.0	19.8	42.5	-0.504	-0.717	74.2
		Control	33.0		25.0		26.2	42.6	34.4		-0.044	-0.227	79.5
	2	PEG	26.0	25.7	13.0	50.0	10.5	20.3	16.8	45.3	0.337	0.123	75.2
		Control	35.0		26.0		17.8	30.6	30.7		0.079		94.7
SW (g/plant)	1	PEG	2.9	25.0	1.8	44.0	0.9	2.5	1.6	56.0	0.039	0.028	55.9
		Control	4.0		3.1		1.5	4.5	3.8		-0.943	0.013	70.8
	2	PEG	3.5	27.3	1.7	57.5	1.0	3.0	2.0	53.5	0.178	0.261	71.9
		Control	5.5		4.0		1.6	5.0	4.3		0.296	-0.542	91.3
RW (g/plant)	1	PEG	0.5	28.0	0.3	50.0	0.2	0.8	0.4	33.3	-0.037	0.02	57.5
		Control	0.7		0.6		0.3	0.9	0.6		0.253	0.345	64.4
	2	PEG	0.7	22.2	0.4	42.9	0.3	0.9	0.4	29.8	0.39	-0.852	79.2
		Control	0.9		0.7		0.4	1	0.6		0.096	0.085	91.3

Correlation coefficients were significantly positive among the three traits under control and PEG conditions in Test 1 and 2 (Table 3). However, the correlation between fresh shoot weight and fresh root weight was the highest (r= 0.602-0.816), followed by the correlation between plant height and fresh shoot weight (r= 0.412-0.617). The correlation between plant height and root weight was the lowest (r= 0.320-0.448).

Table 3. Correlation coefficients among plant height, fresh shoot weight and fresh root weight for Test 1 and 2. The coefficients from the control were represented in upper right side and the coefficients from the PEG treatment were represented in lower left side.

Trait	Test 1			Test 2		
	Plant height	**Shoot weight**	**Root weight**	**Plant height**	**Shoot weight**	**Root weight**
Plant height	-	0.617**	0.448**	-	0.515**	0.410**
Shoot weight	0.412**	-	0.816**	0.480**	-	0.687**
Root weight	0.320**	0.751**	-	0.395**	0.602**	-

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

After three weeks of PEG treatment, all individual plants were also scored for drought tolerance using a drought tolerance index ranging from 0 for a normal growth to 5 for a dead plant (Figure 1 and 2). The results indicated that most of the individual BIL plants were scored as yellowing or wilting cotyledons or true leaves, while some of them were dry or dead due to PEG treatment. Interestingly, a few of them were scored as normal growth with turgid green leaves (i.e., tolerant or resistant to drought). As a comparison, all the plant under the control (water) conditions had normal growth (Figure 1).

Genetic Linkage Mapping and QTL Analysis
Out of 142 BILs used for phenotyping, 93 lines were chosen randomly for marker analysis using 228 AFLP markers, 40 RAPD-AFLP markers, 141 cDNA-AFLP markers, 42 STS markers, and 150 SSR markers. Based on 242 loci assembled onto 41 linkage groups with 2-32 markers per group and a total genetic distance of 842 cM, a total of 7 main effect QTLs were detected for control and PEG conditions (Table 4 and Figure 3). Three QTLs were detected for plant height under control and PEG conditions, each of which explained 16.2- 24.3% of the phenotypic variation, and the superior alleles were all originated from the Pima cotton parent. Two QTLs were detected for fresh shoot weight explaining 13.8- 15.6% of the phenotypic variation: one QTL was from the control and the superior allele was originated from the Upland cotton parent; and the second one was under PEG stress treatment and the superior allele was from Pima S-7. One QTL was detected for fresh root weight under PEG stress conditions and explained 23.8% of the phenotypic variation, and the superior allele was from Pima S-7. One QTL was detected for drought tolerance index and the superior allele was from Upland cotton Sure-Grow 747. Most of the QTLs detected confirmed the pervious notion that Pima cotton is more tolerant to drought stress than Upland cotton. The information for the QTLs is summarized in Table 4.

Table 4. QTLs detected by QTLNetwork2.0 for plant height (PH), fresh shoot weight (SW), fresh root weight (RW), and drought tolerance index (DT), phenotypic variation (PV%), and superior parents under control and PEG conditions.

| Trait	QTL name	Treatment	Interval	Position	Range	Superior parent	PV%
PH	qPH.SP.Ig12-10	Control	1H-590/1F-250	17.5	16.3-18.0	Pima	17.3
PH	qPH.SP.Ig12-14	PEG	1C-290/3F-55	26.3	23.4-28.1	Pima	24.3
PH	qPH.SP.Ig12-20	PEG	1C-125/1F-300	40.4	39.6-42.4	Pima	16.2
SW	qSW.SP.Ig14-1	Control	1H200STS/1H590STS	10	0.0-10.0	Upland	15.6
SW	qSW.SP.Ig12-15	PEG	3F-155/3B-380	29.1	24.4-34.7	Pima	13.8
RW	qRW.SP.Ig.16-2	PEG	1F-480/1F-460	7.2	0.0-7.2	Pima	23.8
DT	qDT.SP.Ig.14-1	PEG	1H200STS/1H590STS	0	0.0-10.0	Upland	13.5
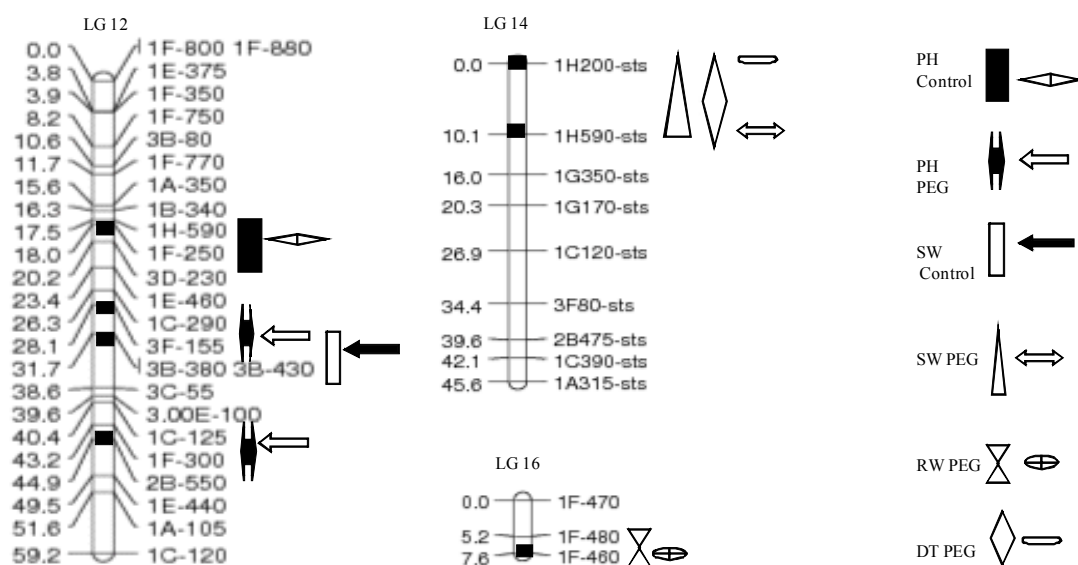
Figure 3. A genetic linkage map of allotetraploid cotton based on SSRs, AFLPs, STS, RAPD-AFLP, and cDNA-AFLP and showing QTLs for drought tolerance.

Summary

PEG (polyethylene glycol) is a non-ionic, long polymer, highly soluble in water. Because of its properties, PEG with high molecular weight has been used to simulate drought stress in plants as a way of lowering water potential in a way similar to soil drying (Larther et al., 1993). So PEG is an important approach to evaluate genotypes for drought tolerance in the greenhouse, because it is easy to maintain humidity and temperature at the vegetative growth phase in the greenhouse (Zhang et al., 2006). This experiment was conducted in the greenhouse to evaluate 142 BILs for drought tolerance using PEG treatment as compared with control conditions. The results showed that significant genotypic variation was detected for plant height, shoot and root weights within the BIL population developed from Upland x Pima. Heritabilities for the three traits were moderate to high and were higher under the control conditions. The three traits were also significantly and positively correlated between each other. Based on a linkage map comprised of 242 loci which were assembled onto 41 linkage groups covering 842 cM, seven QTLs were detected including three for plant height, two for fresh shoot weight, and one for fresh root weight. These QTLs each explained 13.8 to 24.3% of the phenotypic variation and most of superior QTL alleles were from the Pima cotton parent, consistent with its performance under drought stress.

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