



Identification of Drought and Salt Tolerant Cotton Germplasm and Associated Markers in the U.S. Upland Germplasm Pool

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INTRDODUCTION

Abiotic stresses such as drought and salinity prohibit the cotton plant from reaching its maximum genetic potential in productivity. However, due to the complexity in the genetic basis of abiotic stress tolerance and difficulties in phenotyping, information on abiotic stress tolerant cotton germplasm and associated molecular markers is currently lacking.

In recent years, association mapping (AM) has been extensively used in plant genetic studies to dissect complex traits and to identify favorable alleles for traits of interest. AM takes advantage of historic linkage disequilibrium to link phenotypes with genotypes by identifying a statistical association between a change in the DNA sequence and a change in a trait of interest within a population of individuals.

AM or linkage mapping has been conducted in cotton for different traits such as fiber quality; however, only few studies have been performed for drought or salt stress tolerance in cotton.

HYPOTHESIS

Although most if not all of the cotton cultivars and breeding lines released and deposited in the National Plant Germplasm Collection were developed under non-abiotic stress field conditions, desirable genes or alleles with abiotic stress tolerance were randomly fixed in breeding. Cultivars or breeding lines with drought and/or salt tolerance and associated molecular markers can be identified through association mapping.

OBJECTIVES

This study aimed at the dissection of the genetic mechanisms underlying drought and salt tolerance in the panel of U.S. Upland cotton germplasm released up to the mid-2000’s. The collection was chosen to represent a wide range of genetic diversity in Upland cotton. The objectives were,

- to evaluate genetic variation for drought and salt tolerance and traits contributing to abiotic tolerance in the U.S. Upland cotton collection; and
- to detect marker-trait associations for each abiotic tolerance traits, and to identify quantitative trait loci (QTL) affecting drought and salt tolerance in the U.S. Upland cotton that can be used for genetic improvement of abiotic tolerance.

MATERIALS AND METHODS

Cotton germplasm collection

The association panel used in this study consisted of 367 Upland cotton accessions (released up to 2005) collected from 14 different cotton production states in the U.S.

Abiotic tolerance evaluation

The genotypes were evaluated for drought and salt tolerance traits in the greenhouse using a hydroponic system (Abdelraheem et al., 2015a, b). Genotypes were germinated in 2.5-inch plastic pots filled with a commercial soil mix until the second true leaf was emerged. Then individual seedlings were transferred to the hydroponic system containing a half-strength Hoagland's solution for a daily treatment. For salt tolerance treatments, NaCl was added to the container with 50 mM in Day 1, which was increased to 100 mM in Day 2 and 150 mM in Day 3 until the final salinity level of 200 mM NaCl was reached in Day 4. This final concentration was retained for three weeks until seedlings were harvested for growth and physiological measurements. For drought tolerance, 10 % of polyethylene glycol (PEG 8000) was used to treat seedlings and the treatments were also last for three weeks.

The experiment for both abiotic stresses was arranged in a randomized complete block design with 3 replications under treatments (NaCl and PEG) and the same experimental design was used to evaluated the accessions under control conditions with tap water. For each abiotic stress tolerance study, three replicated tests were performed.

Plant measurements

For drought and salt tolerance studies, plant measurements included plant height (PH), shoot fresh (SFW) and root fresh weights (RFW), shoot dry (SDW) and root dry weights (RDW), chlorophyll reading (CC), photosynthesis rate (PSR), evapotranspiration (ET), and leaf temperature (LT) 3 weeks after treatment. Chlorophyll readings were measured with Konica Minolta SPAD-502.

Genotyping

The accessions were genotyped with 500 SSR markers covering 26 chromosomes of the tetraploid cotton genome. This AM panel was studied by Taygi et al. (2014) for its genetic diversity and structure using the SSR markers.

Association mapping

Linkage disequilibrium (LD) was estimated for each pair of SSR loci using TASSEL 2.0.1 software package (www.maizegenetics.net/bioinformatics/tasselindex.htm). LD was calculated using modified D’ method (Hedrick 1987) and tested for a significant P-value for each SSR pair as determined with 1000 permutations.

Mixed linear models (MLMs) and general linear models (GLMs) were used to determine abiotic tolerance trait associated markers using TASSEL 2.0.1 software. The MLM model was performed using Q-matrix and relative kinship among the induvial (K-matrix) (Yu et al., 2006) and a GLM association test was done using Q-matrix.

Linkage map construction

For SSR markers that significantly associated with abiotic stress tolerance, the location and chromosome number were assigned based on the information from Cotton Marker Database (<https://www.cottongen.org/data/markers>). Then the genetic linkage map was constructed using Map Chart2.2(Voorrips, 2002).

RESULTS

Phenotypic variation and heritability estimation

Table 1 shows the results for the analysis of variance (ANOVA) for drought and salt tolerance.

Treatment	S.O.V	D.F	Traits measured after three weeks of treatments								
			PH	SFW	RFW	SDW	RDW	CC	PSR	ET	LT
Salt stress tolerance	Tests (T)	2	4456**	1120**	19.9**	432.8**	98.5**	9560**	78.5**	98**	567**
	Gen. (G)	366	74.0**	11.76**	0.43**	2.43**	0.67**	92.6**	1.3**	2.3**	88.9**
	Salt (S)	1	1580**	950.8**	17.7**	55.65**	53.2**	2134**	35.2**	35**	1345**
	T X G	732	31.3**	0.94**	0.35**	0.35**	5.60**	44.9**	5.60**	5.6**	33.4**
	T X S	2	1210**	238.5**	0.63**	0.63**	0.04**	1635**	3.04**	3.4**	976**
	G X S	366	44.5**	0.95**	0.07**	0.07**	0.03**	57.6**	1.50**	1.9**	31.6**
	T X G X S	732	27.6**	0.70**	0.06**	0.06**	0.07**	33.8**	1.02**	1.2**	21.69**
Drought stress tolerance	Tests (T)	2	2345**	854.4**	14.8**	235.8**	87.6**	6560**	98.5**	77**	367**
	Gen. (G)	366	34.3**	8.43**	0.33**	1.33**	0.55**	77.6**	2.30**	1.9**	76.9**
	Drought (D)	1	1180**	654.2**	15.4**	35.43**	47**	1454**	35.2**	33**	1121**
	T X G	732	14.13**	0.79**	0.21**	0.21**	4.6**	40.6**	5.60**	4.6**	29.4**
	T X D	2	898.5**	123.3**	0.51**	0.51**	0.03**	1435**	3.04**	2.4**	786**
	G X D	366	26.35**	0.69**	0.04*	0.04*	0.02*	54.6**	1.50**	1.5**	28.6**
	T X G X D	732	21.65**	0.54**	0.05**	0.05**	0.06**	29.8**	1.02**	1.2**	20.1**

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

- In the association panel of 367 Upland cotton treated with 10% PEG and 200 mM NaCl using a hydroponic system, a significant reduction in morphological and physiological traits on shoot and root growth due to both treatments was observed, as compared with these same genotypes under the control conditions. There were significant variations (P<0.001) for both shoot and root dry weights and other studied traits.

- The heritability estimates (h²) for the growth traits ranged from 0.39 (plant height under PEG stress conditions) to 0.67 (leaf chlorophyll content under NaCl stress conditions). The heritability estimates for growth traits such as plant height, shoot and root dry weights, leaf chlorophyll content and photosynthesis rate under salt stress were generally higher than these for the same traits under the PEG stress conditions. Significant variations in genotype-by-treatment and genotype-by-test interactions were observed for most of the studied traits.

- The coefficients of variation (CV) ranged from 5.32% (photosynthesis rate) to 43.54% (dry shoot weight) under PEG and from 7.54 % (chlorophyll content) to 55.43% (dry shoot weight) under the NaCl stress conditions. The results indicated that the 376 Upland cotton accessions used in this study represented a higher level of tolerance variation under the NaCl stress treatments than under the PEG stress treatments.

- Furthermore, the results showed that salt tolerance in the association mapping panel was found to be more closely correlated with the biomass produced under the NaCl stress conditions than the biomass produced under the control conditions.

Association analysis

Fig 1 shows common SSR markers associated with the abiotic stress tolerance traits.

- The association of SSR markers with drought and salt tolerance in the AM panel of 367 Upland cotton was tested using the GLM and the MLM models. At the P < 0.05 level, 59 and 71 of 500 SSR markers were found to be associated with drought and salt stress tolerance, respectively. These SSR markers were almost distributed on all the cotton chromosomes. Many SSR markers were associated with more than one trait under PEG and/or NaCl treatments. Most importantly, 12 markers were associated with both drought and salt stress tolerance.

- The phenotypic variation explained (PVE) by each SSR marker ranged from 0.12 % (BNL 3436) to 9.65% BNL 3511), with an average of 3.67 %. Among the 9 traits studied, plant height, fresh shoot weight and chlorophyll content were associated with 29, 27, and 24 under the PEG conditions, while the same traits under the salt conditions were associated with 43, 41, and 37 SSR loci at P <0.05).

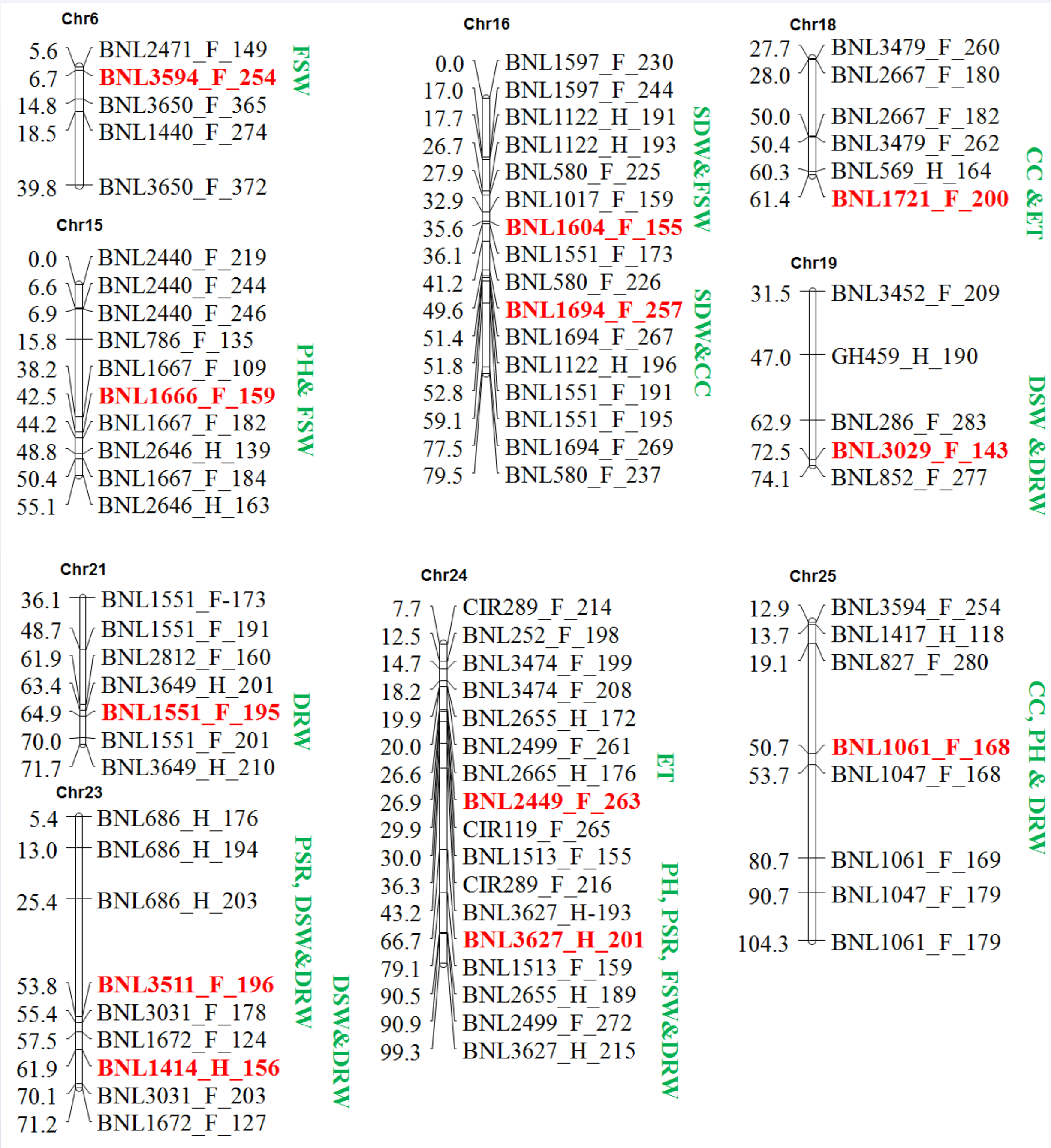
- Based on the genotype data of the loci associated with PEG and NaCl-related traits identified at P<0.05 and the phenotype data of the BLUP results of the 9 PEG and NaCl-related traits, 37 accessions with superior alleles conferring abiotic stress tolerance were identified.

- SSR marker BNL1414 had the highest allelic variation with the maximum positive phenotypic effect (1.23 g/plant) in Deltapine 15 under the salt conditions and in Acala SJ-4 (0.74 g/plant) under the PEG conditions; and SSR marker BNL285 had the maximum negative effect (-0.45 g/plant) in Deltapine 16 under the salt treatment and in Sure-Grow 747 (-1.43 g/plant) under the PEG stress conditions.

- Our results further showed that QTL detected for most of the traits measured under the salt stress conditions had higher P-values and effects than these either under the control or drought stress conditions. The results indicated that selection for salt stress tolerance in cotton under saline conditions at the seedling stage will be useful, consistent with the estimates of heritability for these traits.

RESULTS

Fig 1 SSR markers associated with drought and salt tolerance in the 367 Upland cotton accessions



CONCLUSIONS

An association mapping population of 367 Upland cotton collected from 14 different cotton producing states in the U.S. was genotyped by 500 genome-wide SSRs and phenotyped for drought and salt stress tolerance in the greenhouse. This population revealed abundant phenotypic variations for the traits of interest, and germplasm lines with drought and salt tolerance were identified. Based on the BLUE results, traits such as plant height, fresh shoot weight and chlorophyll content were found to be more closely associated with drought and salt stress tolerance. Therefore, these measurements are good indicators for discriminating tolerance or sensitivity to the abiotic stress conditions in cotton. Moreover, the associated SSR markers and genotypes identified will facilitate the understanding of the genetic basis of drought and salt tolerance in cotton.

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