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Full Length Research Paper

Identification of QTLs and impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from f_6 population using a phylogenetic approach

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Severe drought on cotton plants can slow plant development and cause small bolls and squares to shed. Establishment and prebloom irrigations affect total yield, but water deprivation following bloom and into boll development also affects lint quality. The impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from F_6 population was studied using a phylogenetic approach. It seems that these lines have already evolved different adaptations to drought as a result of their selection environment and it is assumed that different introgression have been stabilized under each environment. Some QTLs were mapped for drought under selected environment, that is, well watered and dryland condition. One QTL (BNL1693) was for seed cotton (SC) on chromosome 1 and 15, while 2 more QTLs (BNL1153 and BNL2884) for SC were identified on Chr6. 3 QTLs, BNL3259, BNL1153 and BNL2884 for osmotic potential were mapped on Chrs 14, Chrs25 and Chr6 respectively. Consistent QTLs for drought resistance traits and yield under drought were detected and can be useful for marker-assisted selection for cotton improvement under drought conditions.

Key words: Cotton, drought, QTLs, environmental effect.

INTRODUCTION

Approximately one third of the world's arable land suffers from chronically inadequate supply of water for agriculture and in virtually all agricultural regions, yield of rainfed crops are periodically reduced by drought (Kramer, 1980; Boyer, 1982). Drought is a major limitation to crop productivity worldwide (Boyer, 1982). For most major food crops, improvement in drought tolerance is an important breeding objective and significant advances have been made over the past 10 - 20 years (Boyer, 1996).The development of drought-tolerant crops has been hindered by lack of knowledge of precise physiological parameters that are diagnostic of genetic potential for improved productivity under water deficit. Many workers in this field were developed over the past couple of decades, covering subjects from plant strategies to control water status under drought (Schulze, 1986a) to the physiological and biochemical processes underlying plant response to water deficits (Chaves, 1991; Cornic and Massacci, 1996). Identifying traits of importance in drought resistance is made difficult by the complexity of climatic variation in precipitation and evapo-transpiration, by the rela-

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tionship between soil moisture status and nutrient availability and by differential plant interactions with this environment. Drought is actually a meteorological event which implies the absence of rain fall for a period of time, long enough to cause moisture deficiency in soil and water deficit with a decrease in water potential in plant tissues (Kramer, 1980). Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological (earliness, reduce leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering).

In genetic sense, the mechanisms of drought resistance can be grouped into three categories, viz. drought escape, drought avoidance and drought tolerance. However, crop plants use more than one mechanism at a time to resist drought. Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits develop. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water-deficit) and remobilization of preanthesis assimilates to grain. Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture, whereas drought tolerance is the ability to withstand water-deficit with low tissue water potential. Mechanisms for improving water uptake, storing in plant cell and reducing water loss confer drought avoidance. The responses of plants to tissue water-deficit determine their level of drought tolerance. Drought avoidance is performed by maintenance of turgor through increased rooting depth, efficient root system and increased hydraulic conductance and by reduction of water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evaporation surface (leaf area). Plants under drought condition survive by doing a balancing act between maintenance of turgor and reduction of water loss. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in cell), increase in elasticity in cell and decrease in cell size and desiccation tolerance by protoplasmic resistance.

In agriculture, drought resistance refers to the ability of a crop plant to produce its economic product with minimum loss in a water-deficit environment relative to the water-constraint-free management. An understanding of genetic basis of drought resistance in crop plants is a pre-requisite for a geneticist to evolve superior genotype through either conventional breeding methodology or biotechnological approach.

Three breeding approaches for drought resistance have been evolved. The first is to breed for high yield under optimum (water-stress-free) condition. As the maximum genetic potential of yield is expected to be realized under optimum condition and a high positive correlation exists between performance in optimum and stress conditions, a genotype superior under optimum level will also yield relatively well under drought condition.

This is the basic philosophy of this approach. However, the concept of expression of maximum genetic potential in optimum condition is debated as genotype environment interaction may restrict the high yielding genotype to perform well under drought. Thus, the second approach, that is, to breed under actual drought condition has been suggested. The second approach suffers from the problem that the intensity of drought is highly variable from year to year and as a consequence environmental selection pressure on breeding materials changes drastically from generation to generation. This problem compounded with low heritability of yield makes for the complicated and slow breeding program. Improving the yield potential of an already resistant material may be a more promising approach, provided there is genetic variation within such a material. Simultaneous selection in non-stress environment for yield and in drought condition for stability may be done to achieve the desired goal of evolving drought-resistant genotype with high yield. On the other hand, biparental mating (half sib and full sib) maintains the broad genetic base and provide the scope to evolve the desirable genotype of drought resistance.

As loss of yield is the main concern for the crop plant from agricultural point of view, plant breeders emphasize on yield performance under moisture stress condition. A drought index which provides a measure of drought based on loss of yield under drought-condition in comparison to moist condition has been used for screening drought-resistant genotype.

The development of drought-tolerant crops has been hindered by lack of knowledge of precise physiological parameters that are diagnostic of genetic potential for improved productivity under water deficit. Using genetic mapping to dissect the inheritance of different complex traits in the same population is a powerful means to distinguish common heredity from casual associations between such traits (Paterson et al., 1988). Although genetic mapping technologies have been available for a decade or more for many major crops, in few cases has it been possible to collect comprehensive phenotypic data both on measures of agricultural productivity and also on differential response among large numbers of genotypes to water deficit. Several investigators have identified quantitative trait loci (QTLs) responsible for improved productivity under arid conditions (Agrama and Moussa, 1996; Tuinstra et al., 1998; Ribaut et al., 1997). Separately, QTLs have also been reported that confer physiological variations that are thought to be associated with stress tolerance.

The objective of present studies were to study the impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from F_6 population using a phylogenetic approach and mapping QTLs for drought under selected environment, that is, well watered and dryland condition.

Table 1. Genotype list 2004 in selected environment (dry land).

par2000	par2001	par2002	Par2003
14	376	206	10
10	469	332	18
33	311	347	20
33	311	26	24
39	451	2	26
28	290	362	35
18	82	341	45
61	388	15	57
44	458	115	62
75	75	63	77
14	376	206	97
14	267	430	101
61	388	15	119
10	469	332	120
18	82	44	123
44	458	433	137
44	458	397	138
18	82	44	183
75	329	443	227
49	176	58	238
75	329	443	276
10	100	255	327
33	311	347	337
14	267	163	338
33	311	26	349
40	154	359	355
39	451	2	358
44	458	115	401
10	100	348	409
8	348	456	439
4	149	396	456
40	154	359	508
26	46	212	513
28	290	362	530
44	458	115	543
75	75	63	547
60	281	326	557
49	115	117	558

Table 2. Genotype list 2004 in selected environment (irrigated land).

par2000	par2001	par2002	par2003
121	672	993	607
110	574	1134	615
146	628	736	642
113	882	854	658
130	817	779	678
120	644	1157	684
103	931	603	706
148	745	638	727
129	720	1001	748
121	672	993	789
110	574	1134	797
123	937	1200	800
107	824	1176	801
103	931	815	805
123	937	620	806
146	726	825	815
103	931	1194	836
127	598	1146	844
123	937	875	880
146	628	869	900
108	612	1062	901
131	914	1014	912
103	931	815	926
148	745	638	933
146	628	736	943
146	628	869	953
123	937	875	958
103	931	1194	960
129	720	1001	1014
108	612	813	1015
132	762	977	1056
120	644	1157	1076
111	775	996	1102
123	937	620	1112
129	965	835	1125
130	817	779	1165
148	745	638	1181
120	644	1157	1188
F-177 (parent 1)			
Vered (parent 2)			

MATERIALS AND METHODS

Population development and selection for contrasting environment

A long term selection program was started (not for breeding purposes but as a preparation for future project) under contrasting environments (dryland - grown on stored water only remember, no summer rain in Israel and well-watered irrigated conditions; Tables 1 and 2). In 2000 F_2 population (Gh x Gb) was grown from which the 38 highest yielding plants were selected under each environment and their F_3 progenies grown fo r further selection under the same environment. In 2001 (12 single plant replicates, 10 reps in subsequent years) again 38 highest yielding plants were selected under each environment (with attention to representing about 20 - 25 F_2 parents) and their progenies grown in the subsequent year and so on up to F_6 lines tested in 2004. Since cotton is a selfer, it was planned at this stage to cross the resulting lines and make segregating populations for further selections. Starting from 2002, twenty out of each set of 38 selected lines were grown not only under their selection environments (for further selection) but also under the contrasting environments (for testing their performance).

Table 3. Productivity of cotton populations selected for lint yield under dry land and irrigated environment.

Year	Population	Selection Environ.	Productivity under the respective environments			
			Seed Cot Yld, g/plant		Lint Yld, g/plant	
			Dry land	Irrigated	Dry land	Irrigated
2002	F_4	dry land	22.26	37.51	8.08	13.82
	F_4	irrigated	16.03	28.61	5.56	10.56
	Statistics		***	*	***	*
	Parental mean		31.22	36.19	12.08	14.08
2003	F_5	dry land	17.31	29.66	6.32	10.23
	F_5	irrigated	19.06	43.54	7.01	15.32
	Statistics		n.s.	**	n.s.	**
	Parental mean		22.65	35.71	8.45	13.72
2004	F_6	dry land	31.58	71.61		
	F_6	irrigated	27.48	72.47		
	Statistics		*	n.s.		
	Parental mean		34.13	63.25		

Microsatellite analysis

Amplification reactions were carried out in 20 uL reaction volumes containing 50 ng genomic DNA, 1.0 µM each of SSR primers sequences which were drawn from the following sources: BNL primers from Brookhaven National laboratory the Research Genetics Co. (Huntsville, AL, USA, http://www.resgen.com); JESPR primers from Reddy et al. (2001), CIR primers from Nguyen et al. (2004) and NAU primers from Han et al. (2004, 2006). 100 uM each of dATP, dCTP, dGTP and dTTP, 1 unit of Taq DNA Polymerase (Fermentas), 1x Taq Polymerase Buffer and 2.5 mM $MgCl_2$. PCR amplifications were performed as described in Zhang et al. (2002), using a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA) programmed as follows: an initial denaturation of 5 min at 94°C; 35 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), and 72°C for 2 min (extension). One additional cycle of 10 min at 72°C was used for final extension. The amplified products were electrophoresed on a 10% nondenatured polyacrylamide gel using a DYCZ-30 electrophoresis apparatus (Beijing WoDeLife sciences instrument company China).

Amplified fragment length polymorphism

AFLP fingerprinting (Zabeau and Vos, 1993; Vos et al., 1995) was performed using Analysis System I (GIBCOBRL Life Technologies) according to the manufacturer's supplied protocol, starting with 225 ng of each genomic DNA. Primary amplifications were performed with 'Mix I' on a Perkin-Elmer 9600 thermocycler. Both the *Eco*RI and *Mse*I primers used in secondary amplification had three extra 'selective' nucleotides at the 3' end menclature follows that of GIBCO-BRL Life Technologies; for example, the primer E-AAG denotes an *Eco*RI cohesive primer with the three selective nucleotides 5'-AAC-3'. The 16 selective primer combinations used in this study. Secondary amplifications containing 33 P labeled *Eco*RI primer were also carried out in a Perkin-Elmer 9600 thermocycler. After the addition of an equal volume (10 µl) of manual sequencing dye (98% formamide, 10 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue), the samples were heated at 94°C for 3 min and chilled on ice. A 2 µl aliquot of each sample was electrophoresed on a denaturing 5% polyacrylamide gel containing 7.5 M urea and a 0.5 × TBE running buffer (45 mM Tris Borate, 1 mM EDTA, pH 8) at 60 W for 1.5 h, in a 30 × 40 cm manual sequencing apparatus (GIBCO-BRL Life Technologies). The gel was transferred to Whatman 3 MM blotting paper, dried under vacuum, and exposed to X-ray film for up to 48 h.

AFLP and SSR data analysis

Since the validity of every polymorphic AFLP band could not be evaluated by an independent method (such as segregation analysis in an F_2 or recombinant-inbred population), only distinct, major, reproducible bands were scored. Minor polymorphic AFLP bands were excluded from the analysis because these can arise artifactually from differences in genomic DNA quality and other factors (Lin and Kuo, 1995; Schondelmaier et al., 1996). Presence or absence of each SSR and AFLP fragment was scored as a binary unit character (1 = present, 0 = absent). Genetic similarities based on Jaccard's coefficient (Jaccard, 1908) were calculated using the SIMQUAL program of the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) Version 2.0 software package (Rohlf, 1993). The resulting genetic similarity matrices were used to generate an unweighted pair group method of arithmetic means (UPGMA) trees (Sokal and Michener, 1958) using the NTSYS-pc. Single factor analysis was performed to find the QTLs in the contrasting environments using T-test sample assuming unequal variance between marker data and yield data.

RESULTS AND DISCUSSION

Plants selected under dryland produced significantly higher yield than those selected under irrigation, both in 2002 and 2004 and no significant difference was found in 2003, with a small but opposite trend (Table 3). Under irrigated conditions, in 2002 plants selected under dryland produced higher yield, in 2003 an opposite (significant) trend was found and in 2004 no difference was found (summary of the results shown in Table 4).

Zeiger during the 90's have shown that Pima cotton bred for yield has evolved heat avoidance via increased transpiration, which is detrimental for water use efficiency (WUE). These findings (supported by other evidences also from Gh cotton) has, presumably, resulted from

Table 4. Seed cotton yield dry and irrigated environment-selection 2004 means.

Dry land		Wet land	
Parent 2003	**Scyield dry**	**Parent 2003**	**Scyield Irri**
10	31.94	607	84.81
18	39.17	615	96.96
20	38.83	642	91.13
24	36.02	658	57.74
26	32.96	678	79.58
35	34.47	684	85.82
45	19.45	706	63.65
57	33.57	727	80.21
62	24.72	748	93.20
77	46.52	789	50.21
97	41.80	797	70.59
101	33.83	800	89.26
119	24.92	801	95.75
120	21.87	805	98.48
123	30.90	806	77.07
137	26.46	815	53.22
138	39.76	836	50.52
183	33.05	844	60.31
227	48.51	880	79.44
238	31.38	900	85.47
276	25.56	901	85.09
327	29.24	912	46.22
337	37.55	926	107.19
338	29.93	933	59.23
349	28.32	943	59.49
355	25.47	953	57.75
358	41.49	958	59.36
401	34.03	960	70.94
409	29.73	1014	56.69
439	25.53	1015	33.62
456	25.31	1056	112.31
508	24.23	1076	51.73
513	29.14	1102	40.93
530	36.46	1112	75.81
543	26.03	1125	69.54
547	22.40	1165	47.49
557	21.36	1181	76.70
558	39.26	1188	100.44

selection and breeding under optimal well-watered conditions. Therefore, it can be hypothesized that selection under drought will lead to different physiological adaptations for drought resistance and WUE. It seems that these lines have already evolved different adaptations to drought as a result of their selection environment and it is assumed that different introgression have been stabilized under each environment. These findings lead to the concept that we can map these lines and identify some interesting introgression present in several dryland selected lines but not in irrigation selected lines.

Collectively 104 SSR and AFLP primers were selected for the present study. These primers were chosen to amplify fragments that were distributed on most of known chromosomes. UPMGA cluster analysis was performed to see is there any impact of selection from various environments on the genetic relationships among the lines. Dendrogram was obtained using SHAN clustering routine

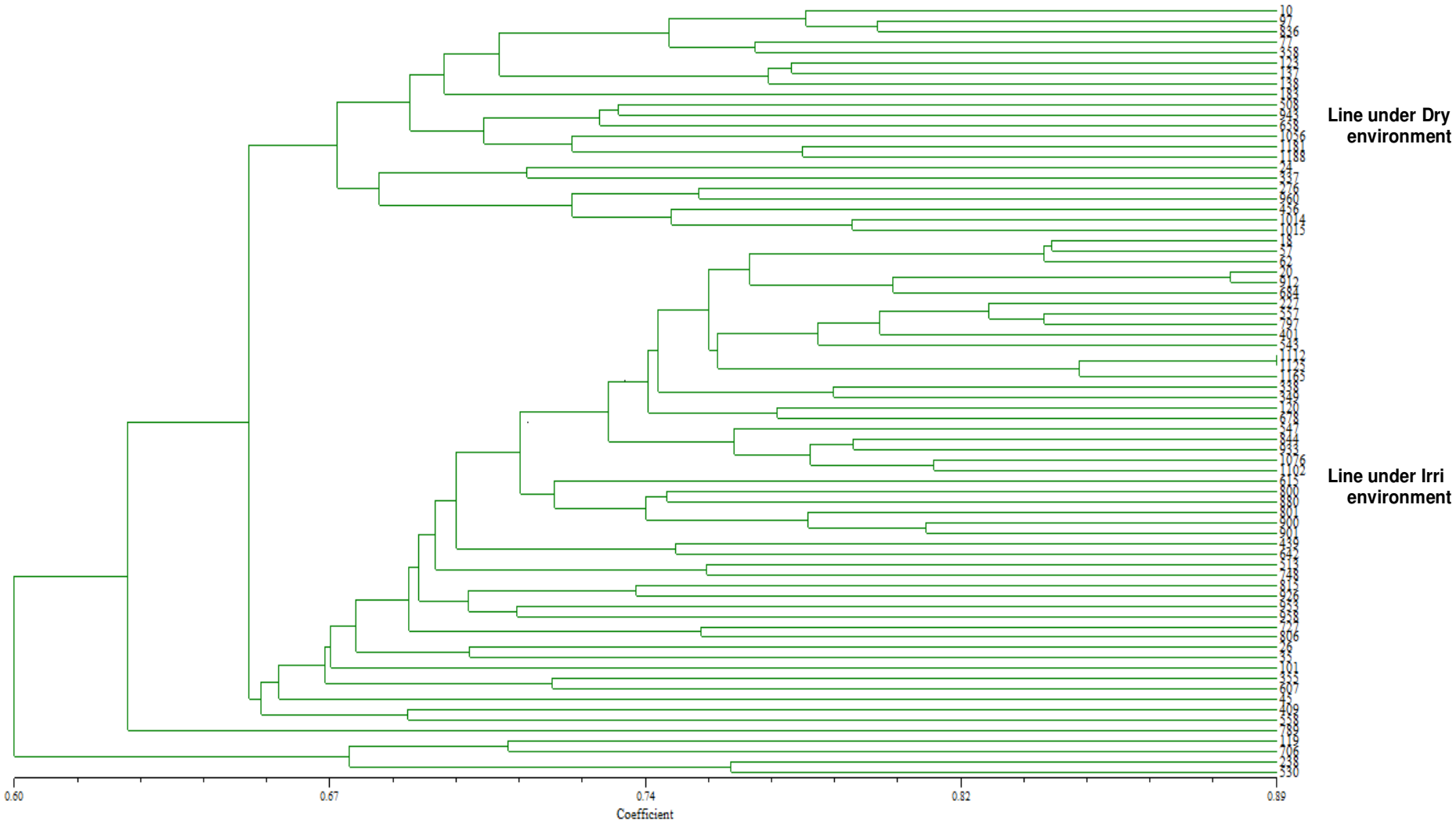

Figure 1. Dendogram showing phylogenetic relationship among 76 cotton plants selected under dry vs irrigated environment using UPGMA method. Scale shows Nei and Li's coefficients of similarity.

of NTSYS-pc (Rohlf, 2004). Dendrogram reveals that as we were looking for small scale differences so we did not find differences in general between two populations Figure 1.

T-test 2 samples assuming unequal variance was per-formed to check the significance of markers with yield data. 19 markers were found to be significant with yield. Altogether 28 SSR and AFLP markers were found to be significant with

Table 5. Cotton drought vs irrigated population data analysis.

Marker	Chrs. #	Correlation Dry vs Irri.	Correlation Yield vs markers	Variance
BNL1693	(Chr.1), Chr.15	0.19	0.396305	0.15705765
BNL3895	Chr.10	-0.1	-0.14302	0.02045472
BNL3955	Chr. 22	-0.1	0.327141	0.10702123
BNL3989	A06	-0.1	-0.17915	0.03209472
STS1206		-0.1	-0.18043	0.03255498
TMHB87-P17		0.37	-0.12009	0.01442160
BNL3031	(Chr.23) Chr.9	0.39	0.260088	0.06764576
AAGb14		-0.1	-0.19109	0.03651538
BNL1227	(Chr.12) Chr.26	0.35	-0.1142	0.01304164
AACb3		0.28	0.223375	0.04989639
AAGb8		0.29	0.192604	0.03709639
BNL3259	Chr. 14	0.37	0.154606	0.02390301
AACb4		0.32	0.415268	0.17244751
ACCb10		0.37	-0.2155	0.04644025
AACb6		0.28	0.313677	0.09839326
AACb5		0.39	- 0.33786	0.11414937
BNL2590	Chr.9	0.46	0.17525	0.03071256
BNL1153	Chr.25, Chr. 6	0.46	0.21592	0.04662144
BNL256	Chr.10	0.46	-0.21367	0.04565486
AACb10		0.41	0.15945	0.03286564
BNL1679	Chr. 12	0.36	0.235869	0.032124
AACb9		0.23	0.14578	0.056321
BNL-2884	Chr. 6	0.12	0.23451	0.03678

yield. List of significant markers and QTLs for drought are presented in Table 5. One QTL (BNL1693) was for seed cotton (SC) on chromosome 1 and 15, while 2 more QTLs (BNL1153 and BNL2884) for SC were identified on Chr6. Three QTLs, BNL3259, BNL 1153 and BNL2884 for Osmotic potential were mapped on Chrs 14, Chrs25 and Chr6 respectively (Table 6).

Simple correlation analysis was conducted using SSR markers data for dry vs irrigated lines. Similarly correlation values for yield vs markers data were calculated. This did not reveal high correlation. Chi-square test was also performed using the proportion of alleles (Table 7). Most of the markers show segregation distortion except TMHB 87-P17 0.92, BNL-3989 0.89 and BNL-3259 with 0.89, this is because of lines selected in the contrasting environments and since these has been established up to F_6. Consistent QTLs for drought resistance traits and yield under drought were detected and can be useful for marker-assisted selection for cotton improvement under drought conditions.

QTLs for drought under dry vs irrigated environments

The extent to which the inheritance of complex traits differs between well-watered and water-limited conditions reflects the complexity of genotype x environment interactions. Our thoughts was to analyzed the data for genetic relationships using a phylogenetic approach to see if there is any impact of selection from various environment on the genetic relationships among the lines. We were looking for difference at the small scale (possibly just one introgression). Therefore, it was possible that two populations may not differ in general (polygeneticly), but yet a small (and important) difference can be observed. Phylogenetic tree did not revealed any differences in general but important differences were observed, since selection of lines were confined to high yield under selected environments, selection pressure for high yield was tends toward the selection of favorable genes for yield hence neglect the region contributing for drought.

We divide the lines based on the 2 selected environments and use a mean separation test to determine any genetic markers showing skewing toward selection environments. Chi-Square test was performed using the proportion of alleles found under the control as an expected value. The difference in phenotype (yield) between the different genotypes for each marker separately (under each environments) was analyzed to confirm association between the allelic diversity and phenotype.

It seems that these lines have already evolved deferent

Table 6. Comparisons of QTLs identification for drought characteristics using single marker analysis.

Marker	P-Value	Map	Drought map		Hua He et al. (2007) Euphytica	
			Chr.	QTL	Chr.	QTL
*BNL1693	0.0000	+	(Chr.1), Chr.15	SC (both)	Chr.1, Chr.15	no
*BNL3895	0.0000	+	Chr.10	DM (wet)	Chr.10	no
*BNL3955	0.0000	+	Chr. 22	no link	Chr.17, chr6	no
*BNL3989	0.0000	+	A06	d13C	Chr.3	no
*STS1206	0.0000					
*TMHB87-P17	0.0000					
*BNL3031	0.0010	+	(Chr.23) Chr.9		Chr.23, chr.9	no
AAGb14	0.0040					
*BNL1227	0.0050	+	Chr.12 Chr.26	not on map	(Chr.12), Chr.26	seed index, lint yield
AACb3	0.0050					
AAGb8	0.0050					
*BNL3259	0.0070	+	Chr. 14	OP	Chr.3	seed per boll
AACb4	0.0100					
ACCb10	0.0470					
AACb6	0.0520					
AACb5	0.0560					
*BNL2590	0.0580	+	Chr.9	no	not on map	no
*BNL1153	0.0670	+	Chr.25, Chr. 6	SC OP	not on map	no
*BNL256	0.0690	+	Chr.10	no	Chr.10, chr25	no
AACb10	0.0780					
*BNL1679	0.0820	+	Chr. 12	not on map	Chr.12	no
AACb9	0.0860					
BNL-2884	0.0880	+	Chr. 6	SC OP	Chr6	no
AACb1	0.0900					
AAGb11	0.0900					
ACCb5	0.0940					
AAGb9	0.1000					
AAGb12	0.1040					

Table 7. Chi-square test.

Ratio	Markers p = 0.05
1:2:1	TMHB 87-P17 = 0.922**
3:1	BNL-3989 = 0.89**
3:1	BNL-3259 = 0.89**
3:1	BNL-1153 = 0.244*
3:1	BNL-2884 = 0.310*

adaptations to drought as a result of their selection environment and it was assumed that different introgression have been stabilized under each environment. It was also believed, that if we map these lines and identify some interesting introgression (present in several dryland selected lines but not in irrigation selected lines) it can pave the way to (1) comparison with "traditional" genetic maps, and (2) further research as to their possible function.

Among a total of 28 QTLs detected 24, 80% showed no significant difference in their effects between well-watered and water-limited conditions, 5 QTLs (3 for productivity and osmotic potential one for dry matter). One QTL influenced productivity, that is, seed cotton yield in both well watered and water-limited treatment. One QTL for dry matter was detected only under well watered conditions influenced the relative values, indicates that partly different sets of genetic loci account for productivity under well-watered versus water-limited conditions.

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