Molecular Breeding of Upland Cotton for Drought Tolerance

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Abstract: Drought stress is a major limiting factor in crop production. Genetic improvement is possible in cotton and other crops against drought stress by molecular breeding exploiting DNA based polymorphism. A drought tolerant (B-557) and a drought susceptible (FH-1000) cultivar were crossed to develop F_2 population. The parents and the F_2 population were studied under osmotic stress in hydroponic culture. A survey of 524 SSR and EST-SSR primers revealed a lot of DNA polymorphism between the drought resistant and drought susceptible cultivar. The polymorphism was used to construct genetic linkage map using the F_2 population. In linkage analysis, 22 primers were mapped on chromosomes. Two QTLs for relative water content were identified on chromosome 23 and 12. One QTL for excised leaf water loss was found on chromosome 23. These QTLs may be used in molecular breeding program to develop drought tolerant cotton cultivars. Positive correlation of relative water content with cell membrane stability reveals that the genes which help plant maintain relative water content may be indirectly involved for cell membrane stability.
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1. Introduction

Drought stress, among abiotic stresses, is the most serious threat to the production of field crops (Loka and Oosterhuis, 2009; Almeselmani et al., 2011). Decreased water availability for agricultural crops demands development of cultivars producing better yield in drought prone environments (Messmer and Stamp, 2010). The genetic ability to withstand drought stress would minimize yield losses. The traits related to drought tolerance in crop plants are complex in nature. However, the traits relative water content, excised leaf water loss and cell membrane stability may be exploited through modern tools like DNA markers (Nguyen, 2000; Jenkins et al., 2001). DNA marker studies have laid foundation to reveal the molecular basis for the traits related to drought tolerance (YongSheng, et al., 2009). Among variety of genetic markers, SSR markers have shown high potential to detect polymorphism (Lin et al., 2010; Dongre et al., 2011) and have been used extensively for cotton genome mapping and marker assisted selection (Frelichowski et al., 2006; He et al., 2007). Researchers have mapped QTLs for morphological traits (Peitong et al., 2005; Liang et al., 2014), physiological traits (Saranga et al., 2004; Saeed et al., 2011), earliness (XianLiang et al., 2008; Li et al., 2013), yield (Babar et al., 2009; LiFang et al., 2010) and fibre traits (Said et al., 2013; Islam et al., 2014).

The improvement in drought tolerance can be enhanced by exploiting certain physiological traits related to drought tolerance. In crop plants during drought stress period, the maintenance of water content in leaves is the most important adaptation (Bartels, 2005; Xoconostel and Ortega, 2010). Relative water content is reported to have significant positive correlation with drought stress tolerance and yield in crop plants (Ciulca et al., 2009; Almeselmani et al., 2011). Lower water loss from leaves help maintain optimum water content in plant. Under stressed conditions, cell membrane stability is affected as the first target of stress (Levitt, 1972). Drought tolerant genotypes tend to maintain integrity of cell membrane under water stress (Bajjii et al., 2001).

Selection of plans for tolerance against stress is very difficult because of genotype × environment interactions (Schuster, 2011). Simulated drought stress in hydroponic conditions using Poly Ethylene Glycol (PEG) has been found effective to evaluate plants because of uniform stress application to populations (Brito et al., 2011; Ren et al., 2011). Present study was conducted for QTL analysis of relative water content, excised leaf water loss and cell membrane stability under simulated drought stress.

2. Material and Methods

A drought tolerant (B-557) and a drought susceptible (FH-1000) genotype selected on the basis of the data for relative water content, excised leaf water loss, cell membrane stability and biomass reduction (manuscript in press) were crossed to develop mapping population. The parental and F_2 populations were evaluated under drought in

hydroponic condition (Fig. 1). Seeds were sown in polythene bags 12″× 4″ filled with sand to develop seedlings. Hoagland solution (Epstein, 1972) was filled in the plastic tank of 2×2 m with 10″ in depth. Ten days old seedlings were placed on Styrofoam sheet and were suspended on Hoagland solution in the tank. There were ten seedlings of each parent and 100 for the F_2 population. Continuous aeration was maintained to the root medium by installing a network of air-pipes connected to an electric motor. Fresh Hoagland nutrient solution was replaced every week. After two weeks, when seedlings proved to be stable in hydroponic culture, plants were exposed to stress by dissolving 15% PEG8000 in the nutrient solution. After one month of stress application, data were recorded for relative water content, excised leaf water loss and cell membrane stability. The plants were gently pulled out from Styrofoam sheet and were placed in oven for dry weight.

Relative Water Content (RWC)

A leaf sample was taken from each plant during early morning. Fresh weight of leaf was recorded immediately after the excision. The samples were kept dipped in water over-night and turgid weight was measured. Then the samples were kept under high temperature (70^{o}C) to record dry weight. The RWC of the leaf sample was calculated by using the following formula as by Clark and Townley-Smith (1986).

RWC = [(Fresh weight-Dry weight) / (Turgid weight-Dry weight)] × 100

Excised Leaf Water Loss (ELWL)

A leaf sample was taken from each plant. The samples were covered with polythene bags soon after excision and fresh weight was recorded using electronic balance. The leaf samples were left on laboratory bench at room temperature. After twenty four hours the weight of the wilted leaf samples was recorded. Then the leaf samples were oven dried at 70°C for recording dry weight. Excised leaf water loss was calculated using the following formula as by Clarke and McCaig (1982).

ELWL = [(Fresh weight-Wilted weight) / Dry weight]

Cell Membrane Stability (CMS)

A leaf sample was taken from each plant. The samples were rinsed with deionized water to remove surface contamination. Leaf discs of 10mm diameter were taken in falcon tubes with six discs in each tube. Tubes were filled with 20 ml deionized water and kept at room temperature for two hours and after shaking an initial conductance (C_1) reading was made. Then samples were autoclaved at 121^{o}C for 15 minutes and were kept at room temperature for overnight to take second conductance (C_2) reading. The CMS of the leaf discs was calculated as reciprocal of relative cell injury as by Saadalla *et al.* (1990) and modified by Petcu and Terbea (1995).

% injury = $(C_1/C_2) \times 100$

Cell membrane stability was calculated using the formula:

CMS = 1- % injury = 1 - $(C_1/C_2) \times 100$, where C_1 and C_2 are the first and the second reading of conductance repectively.

Molecular Work

The leaves of the F_2 and parent plants were used for DNA extraction. Leaves were detached, packed in plastic bags and immediately transferred to freezer -80^{o}C. Standard CTAB method (Doyle and Doyle, 1990) was used for DNA extraction. The parents, FH-1000 and B-557 were screened with 524 pairs of SSR primers to identify polymorphic primers. The primers of different series (NAU, DPL, JESPR, CIR, BNL, CTM and MUCS) were selected in a way to cover the whole genome. PCR products were run on 10% polyaccrylamide gels using Bio Rad Gel apparatus, followed by Silver Nitrate Staining. One hundred F_2 plant DNA sample were screened with 44 polymorphic SSR primers. The segregation ratio 3:1 (dominant marker) or 1:2:1 (co-dominant marker) was assessed with chi-square test for goodness of fit. The size of bands developed from almost all primers was same as was reported in cotton marker database.

Linkage Analysis:

Linkage software Joinmap3.0 (Van-Ooijen and Voorrips, 2001) was used for the analysis. The Kosambi mapfunction (Kosambi, 1944) was used to convert recombination frequency to genetic map distance in centi Morgan (cM). Band scoring was conducted by following the instruction given in manual of the software.

1 = Genotypes of parent A (B-557)
2 = Genotypes of parent B (FH-1000)
3 = Heterozygote
Other situations were coded by:
4 = Not A; i. e. 3 or 2 (for dominant markers)
5 = Not B; i. e. 3 or 1 (for dominant markers)
'-' = Missing data for the individual at a locus

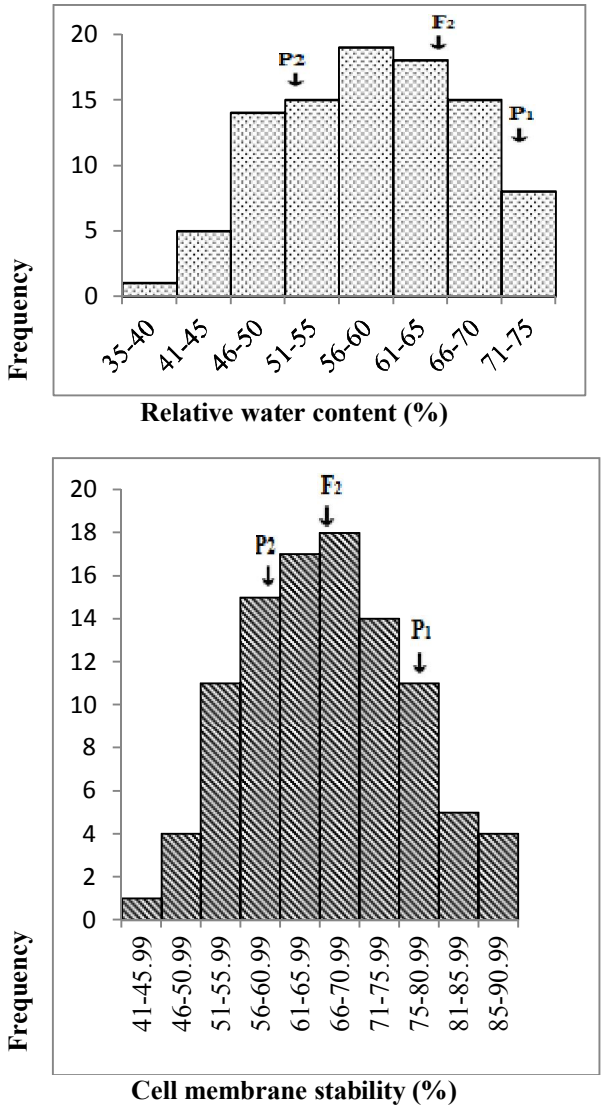
QTLs Mapping

Marker and QTL association analysis for the traits related to drought tolerance was carried out by using software QTL cartographer2.5 (test statistics composite interval mapping CIM). Data for input files (linkage map, molecular marker and phenotypic data) was prepared according to the instructions given in the manual (Basten et al., 2001; Van-Ooijen and Voorrips, 2001). The proportion of observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from the corresponding model (Basten et al., 2001) for analysis. Permutation-1000 test ($P < 0.05$) was

performed to determine threshold LOD value to declare a QTL.

3. Results

Fig. 1: Evaluation of the parents and the F2 population of the cross B-557 × FH-1000 in hydroponic culture



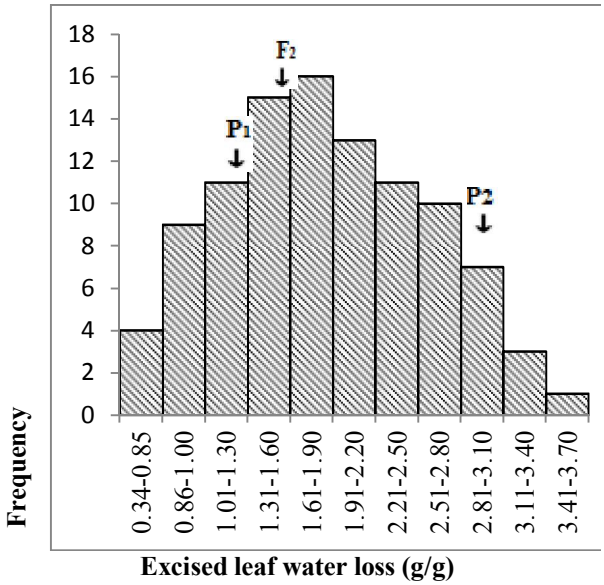


Fig. 2: Frequency distribution for relative water content, excised leaf water loss and cell membrane stability F_2 population of cross B-557 × FH-1000 evaluated in hydroponic culture.

Significant differences were observed among the parental and F_2 generation ($P < 0.01$) for the traits, relative water content, excised leaf water loss, cell membrane stability and plant dry weight. The F_2 population for the traits showed normal distribution revealing quantitative inheritance (Figure 2), which suggests that the traits were suitable for QTL analysis (Jenkins et al., 2001). Relative water content had positive correlation with cell membrane stability, whereas, excised leaf water loss had negative correlation with cell membrane stability (Table 1).

Table 1: Phenotypic correlation among the traits excised leaf water loss (ELWL), relative water contents (RWC), cell membrane stability (CMS, %) and plant dry weight (PDW) in F_2 population of the cross B-557 × FH-1000 in hydroponic culture.

	ELWL	RWC	CMS
RWC	-0.22		
CMS	-0.26**	0.59**	
PDW	-0.19	0.49**	0.53**

* = P < 0.05, ** = P < 0.01

The SSR analysis in the present study revealed 8.39% polymorphic primers between the parents (Figure 3). Among different primer series, the SSR series NAU was more polymorphic (11.01%). One hundred F_2 plants were screened with 44 polymorphic SSR primers (Figure 4). Linkage analysis resulted in mapping of 22 primers (LOD 3) in 8 linkage groups, covering a total of 264 cM, which is 5.64% of allotetraploid cotton genome. The linkage groups (LG) were arranged in ascending order of length (LG1-LG8) and chromosomes were assigned by using information from cotton marker database (Table 2). Linkage groups LG1, LG2, LG3, LG4, LG5, LG6 and LG7 were assigned to chromosome 25, 23, 12, 11, 3, 6 and 13 respectively. The linked markers were used for QTLs mapping for the traits related to drought tolerance in cotton. A total of 8 linkage groups were generated which covered 264 cM, and is 5.64% of total 4660 cM recombinational length of cotton genome. Two QTLs (*qtl*RWC-1 and *qtl*WC-2) were detected for relative water content (Table 3). The nearest marker of *qtl*RWC-1 was NAU2954 assigned to chromosome 23. For *qtl*RWC-2, the nearest marker was NAU2715, assigned to chromosome 12. The phenotypic variance (R^2) for *qtl*RWC-1 and *qtl*RWC-2 was 13% and 68% respectively. The positive value of additive effect for both QTLs represents that the alleles were contributed by tolerant parent (B-557). One QTL (*qtl*ELWL) was detected for the trait, excised leaf water loss under drought stress. Nearest marker for this QTL was NAU2954, assigned to chromosomes 23. The phenotypic variance (R^2) for this trait was 63%. Negative value of additive effect represents that the alleles for this trait were contributed by susceptible parent (FH-1000).

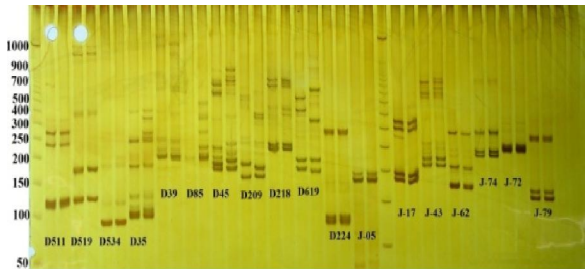


Fig. 3: Parental screening with SSR primers of different series (NAU, DPL, JESPR)

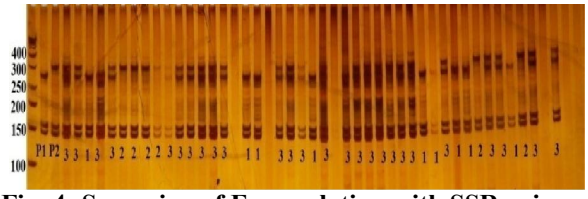


Fig. 4: Screening of F_2 population with SSR primer NAU-2954

Table 3: The QTLs detail for the traits, relative water content (RWC) and excised leaf water loss (ELWL)

Trait Name	QTL	Nearest Marker	LOD	R^2	Chr. No	Position (cM)
RWC	1	NAU2954	2.74	0.13	23	115.3
	1	NAU2715	10.91	0.68	12	56.6
ELWL	1	NAU2954	11.99	0.63	23	115.3

4. Discussions

Relative water content is measurement of plant water status in a given environment and has been reported to be correlated with drought stress tolerance and yield in crop plants (Ciulca et al., 2009; Almeselmani et al., 2011). Leaf water contents have direct effect on cellular membrane integrity. Loss of leaf turgor causes dehydration in cell and eventually cell membrane lose integrity. In the present study, positive correlation of relative water content and cellular membrane stability depicts that the plant with higher water content may maintain cellular membrane integrity under drought stress Cell membrane stability has also been reported to be associated with drought tolerance and yield in crop plant (Almeselmani et al., 2011) and has been considered as an important selection criterion of drought tolerance in cotton (Rahman et al., 2006, Azhar et al., 2009) The negative correlation of excised leaf water loss with cell membrane stability depicts that lower water loss from

leaves help maintaining relative water content and hence cell membrane stability. Excised leaf water loss is also considered as drought tolerant trait in crop plants (Clarke and Townley-Smith, 1986; Winter et al., 1988).

Compared to other crops, cotton has a low genetic variation (Chee et al., 2004; Lubbers et al., 2004). Upland cotton grown in the world is selection from four varietal types namely Acala, Stoneville, Coker and Deltapine; Coker, Deltapine and Stonville with a common ancestor (Niles, 1980). In the present study 8.39% polymorphism was observed between cotton parents. Similarly, Frelichowski et al. (2006) found 11.3% and Wang et al. (2006) observed 3.1% intera-specific polymorphism. Although, majority of linkage map has been constructed by using the mapping population developed from inter-specific crosses but these have little importance in breeding programmes. Wu et al. (2009) indicated that the marker identified from intra-specific cross could be useful in marker assisted breeding for cotton. Intera-specific (*G. hirsutum* × *G. hirsutum*) population of cotton has been used for construction of linkage map by many researchers (LiFang et al., 2010; Saeed et al., 2011). The best confidence interval proposed for QTLs mapping is 10 cM (Kearsey, 1998). In this study 8 linkage groups were resulted with an average length of 19.14 cM. Seven groups were assigned to seven chromosome based on data available for assigning SSRs to chromosomes by linkage analysis (Lacape et al., 2003; Nguyen et al., 2004).

The identification of the genomic regions associated with physiological traits related to drought tolerance have been reported in many crops such as rice (Courtois et al., 2000; YanYing et al. 2008), maize (Rahman et al., 2011), barely (Teulat et al. 2001), soybean (Virginia et al., 2012) and wheat (Ciuca and Elena, 2009). In cotton, a few studies have been conducted for physiological traits (Saranga et al., 2004; Saeed et al., 2011). The QTLs for relative water content and excised leaf water loss has been detected for the first time in the present study**.**

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Conclusion:

The study concludes that the traits, high relative water content, lower excised leaf water loss and cell membrane stability are good indicators of drought tolerance in cotton. The QTL detected for the traits have revealed the genetic basis of the traits so cotton breeders may exploit these traits to engineer drought tolerant cultivars.

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