

QTL MAPPING FOR SOME IMPORTANT DROUGHT TOLERANT TRAITS IN UPLAND COTTON

M. A. Saleem, T. A. Malik, A. Shakeel and M. Ashraf¹

Dept. of Plant Breeding and Genetics, ¹Dept of Botany, University of Agriculture Faisalabad, Faisalabad.
Corresponding Author email address: asifsaleempbg@gmail.com

ABSTRACT

Drought stress is a major factor limiting crop production. Genetic improvement is possible in cotton and other crops against drought stress by molecular breeding. A drought tolerant (B-557) and a drought susceptible (FH-1000) cultivar were crossed to develop F₂ population. The parents and the F₂ 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 F₂ population. In linkage analysis, 22 primers were mapped on chromosomes. Two QTLs for relative water content were identified. One QTL was mapped on chromosome 23 with nearest marker NAU2954 and another QTL was mapped on chromosome 12 with nearest marker NAU2715. One QTL for excised leaf water loss was found on chromosome 23 with nearest marker NAU2954. These QTLs may be used in molecular breeding program to develop drought tolerant cotton cultivars.

Key words: Cotton, drought, relative water content, excised leaf water loss, cell membrane stability, QTL.

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 such as 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 drought tolerant traits such as relative water content, excised leaf water loss and cell membrane stability..

MATERIALS AND METHODS

A total of thirty genotypes/cultivars (20 drought tolerant and 10 susceptible) were collected from different research stations of Pakistan. 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 F₂ 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 F₂ 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°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).

$$\text{RWC} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100$$

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).

$$\text{ELWL} = [(\text{Fresh weight} - \text{Wilted weight}) / \text{Dry weight}]$$

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 1.0 cm² were sliced from samples and were submerged in 10 ml deionized water in 20 ml screw-cap vials which were kept at room temperature in dark for 24 hours. Conductance of the solution was measured with a conductivity meter (Jenway modal 4070). The vials were then autoclaved for 15 minutes and conductance of the sample solutions was measured again to estimate electrolyte concentration. All measurements were recorded at 25 °C by keeping vials submerged in a water bath. The CMS of the leaf discs was calculated as reciprocal of relative cell injury (Blum and Ebercon, 1981) using the following formula:

$$\text{CMS\%} = \{[1 - (T1/T2)] / [1 - (C1/C2)]\} \times 100$$

Where, T1= Stress sample conductance before autoclaving.

T2= Stress sample conductance after autoclaving.

C1= Control sample conductance before autoclaving.

C2= Control sample conductance after autoclaving

DNA Extraction: The leaves of the F₂ and parent plants were used for DNA extraction. Leaves were detached, packed in plastic bags and immediately transferred to freezer at -80°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 were selected in a way to cover the whole genome (Table 1). PCR products were run on 10% polyacrylamide gels using Bio Rad Gel apparatus, followed by Silver Nitrate Staining. One hundred F₂ 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 (R²) 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.

RESULTS AND DISCUSSION

Significant differences were observed among the parental and F₂ generation (*P* < 0.01) for the traits, relative water content, excised leaf water loss, cell

membrane stability and plant dry weight. The F₂ population for the traits showed normal distribution (Fig. 4) revealing quantitative inheritance, which suggests that the traits were suitable for QTL analysis (Jenkins *et al.*, 2001)

The correlation matrix of the traits is given in the Table 1. Excised leaf water loss had negative correlation with cell membrane stability. Relative water content correlated positively with cell membrane stability. 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).

A total of 524 SSR and EST-SSR primers were used to screen the parents (B-557 and FH-1000) for polymorphism (Fig. 2). Primers of the series NAU, DPL, JESPR, CIR, BNL, CTM and MUCS were used to observe polymorphism between the parents. The SSR series NAU was more polymorphic (11.01%). A total of 44 unambiguous polymorphic SSR primers were selected for screening of F₂ population. The SSR analysis in the present study revealed 8.39% polymorphic primers between the parents. Frelichowski *et al.* (2006) found 11.3% and Wang *et al.* (2006) observed 3.1% inter-specific polymorphism. 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).

One hundred F₂ plants were screened with 44 polymorphic SSR primers (Fig. 3). The segregation ratio 3:1 (dominant marker) or 1:2:1 (co-dominant marker) was assessed with chi-square test for goodness of fit. A total of 44 unambiguous polymorphic (easy to score allele) SSR primers were used to construct a linkage map. 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 (Table 3). 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. 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. Inter-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). One group LG8 could not be assigned to any chromosome.

Two QTLs (*qtLRWC-1* and *qtLWC-2*) were detected for relative water content (Table 3). The nearest marker of *qtLRWC-1* was NAU2954 assigned to chromosome 23. For *qtLRWC-2*, the nearest marker was NAU2715, assigned to chromosome 12. The phenotypic variance (R²) for *qtLRWC-1* and *qtLRWC-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 (*qtLELWL*) 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 (R²) for this trait was 63%. Negative value of additive effect represents that the alleles for this trait were contributed by susceptible parent (FH-1000).

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.

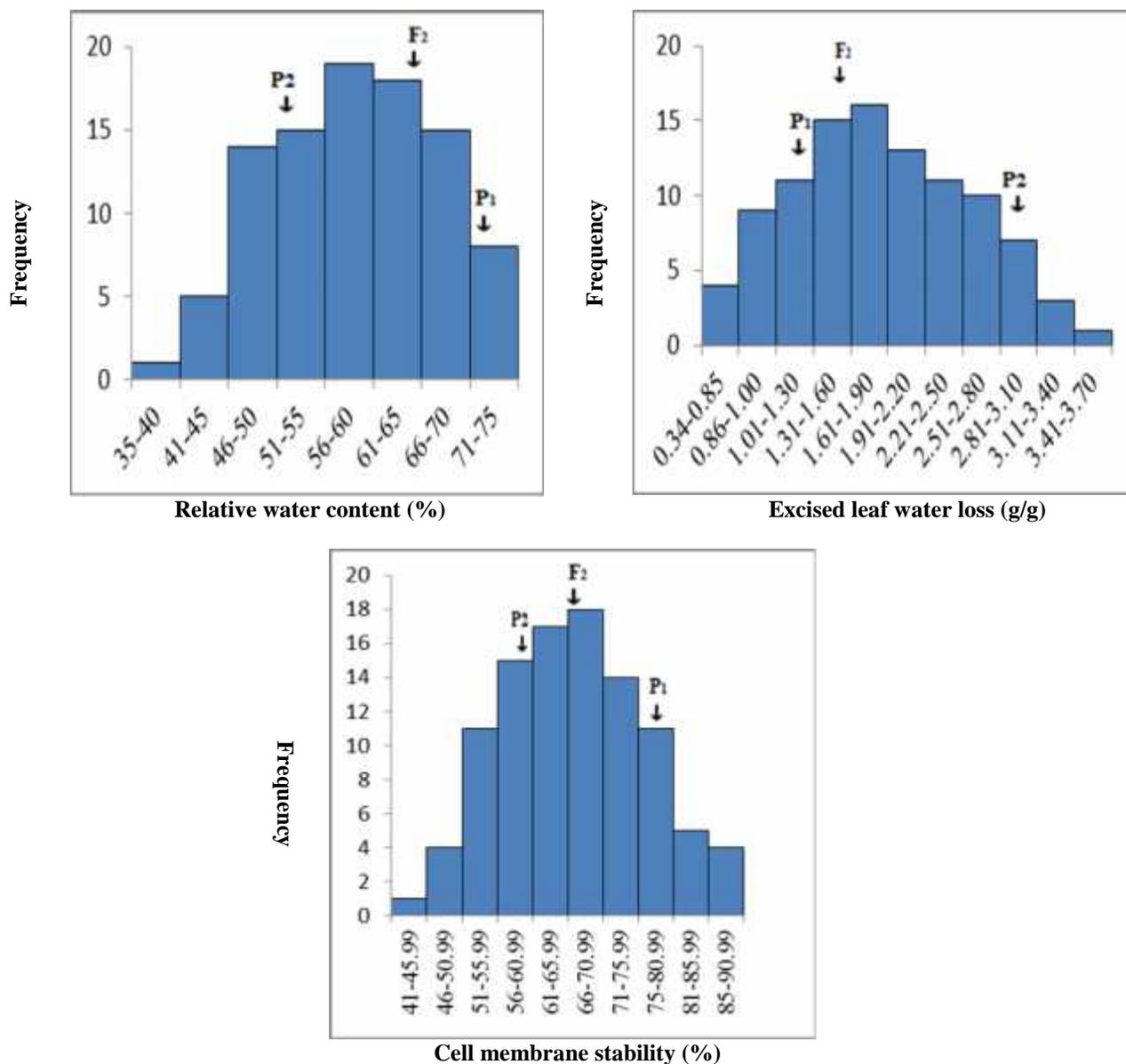


Fig. 4. Frequency distribution for relative water content, excised leaf water loss and cell membrane stability in F₂ population of cross B-557 × FH-1000 evaluated in hydroponic culture.

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 F₂ 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

Table 2. Linkage Analysis of Primers with the Chromosomes.

Linkage Group	Primers	Chromosome
1	DPL519 DPL323 NAU2838	25
2	NAU2954 JESPR101	23
3	NAU2715 NAU2868	12
4	DPL0209 DPL0675 JESPR135 DPL270	11
5	DPL733 NAU862 JESPR231	3
6	NAU2967 NAU5269 NAU3427	6
7	NAU3203 CIR096 JESPR153	13
8	NAU6105 NAU6109	-

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

Trait Name	QTL/QTLs	Nearest Marker	Additive Effect	LOD	R ²	Chromosome	Position (cM)	Size (bp)
RWC	1	NAU2954	4.87	2.74	0.13	23	115.3	150
	1	NAU2715	6.44	10.91	0.68	12	56.6	190
ELWL	1	NAU2954	-0.23	11.99	0.63	23	115.3	150

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.

Acknowledgment: This study was part of the PhD project entitled: Genetics and DNA marker studies for drought tolerance in upland cotton. The authors are thankful to Higher Education Commission of Pakistan for providing grant. Guidance of Dr. Pang W. Chee, Cotton Molecular Lab., University of Georgia for PCR analysis is also highly acknowledged.

REFERENCES

- Almeselmani, M., F. Abdullah, F. Hareri, M. Naesan, M.A. Ammar, O.Z. Kanbar and A. Saud (2011). Effect of drought on different physiological characters and yield component in different Syrian durum wheat varieties. *J. Agric. Sci.* 3 (3): 127-133.
- Azhar, F.M., Z. Ali, M.M. Akhtar, A.A. Khan and R. Trethowan (2009). Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). *Plant Breed.* 128: 356-362.
- Babar, M., Y. Saranga, Z. Iqbal, M. Arif, Y. Zafar, E. Lubbers and P. Chee (2009). Identification of QTLs and impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from F₆ population using a phylogenetic approach. *African J. Biotech.* 8: 4802-4810.
- Bajjii, M., J.M. Kinet and S. Lutts (2001). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Reg.*: 1-10.
- Bartels, D. (2005). Dessication tolerance studied in resurrection plant *Craterostigma plantagineum*. *Integrative Comp. Biol.* 45: 696-701.

- Basten, C.J., B.S. Weir and Z.B. Zeng (2001). QTL cartographer, version 1.15. Raleigh, NC, Department of Statistics, North Carolina State University, NC, USA.
- Blum, A. and A. Ebercon (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21: 43-47.
- Brito, G.D., V. Sofiatti, M.D.A. Lima, L.P.D. Carvalho and J.L.D.S. Filho (2011). Physiological traits for drought phenotyping in cotton. *Acta Scientiarum. Agro.* 33: 117-125.
- Chee, P.W., J. Rong, C.D. Williams, S.R. Schulze and A.H. Paterson (2004). EST derived PCR-based markers for functional gene homologues in cotton. *Genome*, 47: 449-462.
- Ciuca, M. and P. Elena (2009). SSR markers associated with membrane stability in wheat (*Triticum aestivum* L.). *Romanian Agric. Res.* 44: 303-307.
- Ciulca, S., E. Madosa, A. Ciulca, G. Velicevici and S. Chis (2009). Evaluation of drought tolerance in winter barley using different screening techniques. *Genetika*, 3: 953-978.
- Clarke, J.M. and T.F. Townley-Smith (1986). Heritability and relationship to yield of excised leaf water retention in durum wheat. *Crop Sci.* 26: 289-292.
- Clarke, J.M. and T.N. McCaig (1982). Excised leaf water retention capability as an indicator of drought resistance of *Triticum* genotypes. *Can. J. Plant Sci.* 62: 571-578.
- Courtois, B., G. McLaren, P.K. Sinha, K. Prasad, R. Yadav and L. Shen (2000). Mapping QTLs associated with drought avoidance in upland rice. *Mol. Breed.* 6: 55-66.
- Dongre, A.B., M.P. Raut, V.M. Paikrao, R.A. Kadam and S.S. Ashtikar (2011). Use of RAPD and ISSR markers for determining genetic diversity of cotton (*Gossypium hirsutum* L.) working germplasm. *J. Cotton Res. Dev.* 25: 137-143.
- Doyle, J.J. and J.L. Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15
- Epstein, E (1972). Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, New York, USA.
- Frelichowski, J.E., M.B. Palmer, R.J. Kohel and M. Ulloa (2006). Cotton genome mapping with new microsatellites from Acala 'Maxxa' Bac-ends. *Mol. Genet. Genome*, 275: 479-491.
- He, D., X. Lin and W. Li (2007). QTL mapping for economic traits based on a dense Genetic map of cotton with PCR-based markers using the interspecific cross of *Gossypium hirsutum* and *Gossypium barbadense*. *Euphytica*, 53: 181-197.
- Islam M. S., Z. Linghe, D. D. Christopher, S. Xianliang, J. K. Hee, L. Ping and D. D. Fang (2014). Identification of cotton fiber quality quantitative trait loci using intraspecific crosses derived from two near-isogenic lines differing in fiber bundle strength. *Mol Breed.* DOI 10.1007/s11032-014-0040-4
- Jenkins J. N., Z. W. Shappley, J. Zhu and J. C. McCarty (2001). Molecular markers and quantitative traits in *Gossypium hirsutum* L. In. "Genetic improvement of cotton, emerging technologies", ed. J. N. Jenkins and S. Saha. Oxford and IBH publishing co. New Delhi, India.
- Kearsey, M.J. (1998). The principles of QTL analysis (a minimal mathemataics approach). *J. Exp. Bot.* 49: 1619-1623.
- Kosambi, D.D. (1944). The estimation of map distance from recombination values. *Ann. Eugenics*, 12: 172-175.
- Lacape, J.M., T.B. Nguyen, S. Thibivilliers, T.B. Bojinnov, B. Courtois, R.G. Cantrell, B. Burr and B. Hau (2003). A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* x *Gossypium barbadense* backcross population. *Genome*, 46: 612-626.
- Levitt, J. (1972). Responses of plants to environmental stresses. Academic press, New York.
- Li., X.W., D. Na, Z. Haihong, X. Zhe, W. Rui, L. C. Richard and Q. Wang (2013). QTL analysis for early-maturing traits in cotton using two upland cotton (*Gossypium hirsutum* L.) crosses. *Breed. Sci.* 63: 154-163
- Liang Q., P. Li, C. Hu, H. Hua, Z. Li, Y. Rong, K. Wang and J. Hua (2014). Dynamic QTL and epistasis analysis on seedling root traits in upland cotton. *J. Genet.* 93: 63-78
- LiFang, S., H. Lei, H. BaoMin, C. Ling and W. PeiZheng (2010). QTL mapping of yield and agronomic traits of interspecific hybrid cotton. *Xinjiang Agric. Sci.* 47: 67-72.
- Lin, Z., D. Yuan and X. Zang (2010). Mapped SSR markers unevenly distributed on the cotton chromosomes. *Front. Agric. China*, 4: 257-264.
- Loka, D.A. and D.M. Oosterhuis (2009). Effect of water-deficit stress on reproductive development in the cotton pistil. *Summaries of Arkansas Cotton Research. AAES Research Series:* 582.
- Lubbers E., P. Chee, J. Gannaway, R. Wright, K. El-Zik and A.H. Paterson (2004). Levels and patterns of genetic diversity in upland cotton. In: *Plant and Animal Genome XII Conference*, San Diego, USA.
- Messmer, R. and P. Stamp (2010). Trends in drought research. *Kasetsart J. Natural Sci.* 44: 507-516.

- Nguyen, H.T. (2000). Molecular Dissection of Drought Resistance in Crop Plants: from Traits to Genes. CIMMYT, Mexico.
- Nguyen, T.B., M. Giband, P. Brottier, A.M. Risterucci, and J.M. Lacape (2004). Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. *Theor. Appl. Genet.* 109: 167-175.
- Niles, G.A. (1980). Breeding cotton for resistance to insects pests. In "Breeding plants resistance to insects", ed. F.G. Maxwell and P.R. Jennings. John Willy and Sons, New York, USA.
- PeiTong, Z., Z. XieFei, G. WangZhen and Z. TianZhen (2005). Genetic analysis and QTLs tagging of lint percentage and its closely related yield components in upland cotton. *Iangsu J. Agric. Sci.* 21: 264-271.
- Rahman M., I. Ullah and Y. Zafar (2006). Genotypic variation for drought tolerance in cotton (*Gossypium hirsutum* L.): seed cotton yield responses. *Pakistan. J. Bot.* 38: 1679-1687.
- Rahman, H., S. Pekic, V.L. Jancic, S.A. Quarrie, S.M.A. Shah, A. Pervez and M.M. Shah (2011). Molecular mapping of quantitative trait loci for drought tolerance in maize plants. *Genet. Mol. Res.* 10: 889-901.
- Ren, S., S. Weeda, O. Akande, Y. Guo and L. Rutto (2011). Drought tolerance and AFLP-based genetic diversity in purslane (*Portulaca oleracea* L.). *J. Biotech. Res.* 3: 51-61.
- Said, J. I., L. Zhongxu, Z. Xianlong, S. Mingzhou and J. Zhang (2013). A comprehensive meta QTL analysis for fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton. *BMC Genomics.* 14:776
- Saeed, M., W. Guo, I. Ullah, N. Tabbasam, Y. Zafar, Mehboob-ur-Rahman and T. Zhang (2011). QTL mapping for physiology, yield and plant architecture traits in cotton (*Gossypium hirsutum* L.) grown under well-watered versus water-stress conditions. *Electronic J. Biotech.* 1-13.
- Saranga, Y., C.X. Jiang, R.J. Wright, D. Yakir and A.H. Paterson (2004). Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. *Plant Cell Env.* 27: 263-277.
- Schuster, I. (2011). Marker-assisted selection for quantitative traits. *Crop Breed. Appl. Biotech.* 11: 50-55.
- Teulat B., C. Borries and D. This (2001). New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. *Theor. Appl. Genet.* 103: 161-170.
- Van-Ooijen, J.W. and R.E. Voorrips (2001). JoinMap @ Version 3.0: software for the calculation of genetic linkage maps. Wageningen, CPRO-DLO, Netherland.
- Virginia, S., M. Pagan, M. Cooper, S.K. Kantartzi, D.A. Lightfoot, K. Meksem and M.A. Kassem (2012). Genetic analysis of relative water content (RWC) in two recombinant inbred line populations of soybean [*Glycine max* (L.) Merr.]. *J. Plant Genome Sci.* 1: 46-53.
- Wang, B., Y. Wu, N. Huang, X. Zhu, W. Guo and T. Zhang (2006). QTL mapping for plant architecture traits in upland cotton using RILs and SSR markers. *Acta Genetica Sinica*, 33: 161-170.
- Winter, S.R., J.T. Musick and K.B. Porter (1988). Evaluation of screening techniques for breeding drought-resistant winter wheat. *Crop Sci.* 28: 512-516.
- Wu, J.X., O.A. Gutierrez, J.N. Jenkins, J.C. McCarty and Z. Jun (2009). Quantitative analysis and QTL mapping for agronomic and fiber traits in an RIL population of upland cotton. *Euphytica*, 165: 231-245.
- XianLiang, Z., W. KunBo, S. GuoLi, L. Fang, L. ShaoHui, W. ChunYing, Z. XiangDi and W. YuHong (2008). QTL mapping of upland cotton RIL CRI-G6 by SSR marker. *Cotton Sci.* 20: 192-197.
- Xoconostel, C.B. and F.A.R. Ortega (2010). Drought tolerance in crop plants. *American J. Plant Physiol.* 5: 241-256.
- YanYing, Q., M. Ping; L. XueQin, T. YuXiu, W. Feng, Z. HongLiang and L. ZiChao (2008). QTL mapping and correlations between leaf water potential and drought resistance in rice under upland and lowland environments. *Acta Agronomica Sinica*, 34: 198-206.
- YongSheng, Q., L. RenZhong, M. HongXian, Z. TianZhen and G. WangZhen (2009). QTL mapping for yield traits in Upland cotton (*Gossypium hirsutum* L.). *Acta Agronomica Sinica*, 35: 1812-1821.