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Molecular characterization of tolerant and susceptible accessions of cotton (*Gossypium hirsutum* L.) against waterlogging stress

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Abstract : Waterlogging in the cotton ecosystem causes greater yield losses. Based on morphological, anatomical, physiological and biochemical analysis, germplasm accessions such as IC359979, IC359245, IC357235, IC563998and IC357558, IC359242, IC357607, IC356708 were identified as tolerant and susceptible respectively from seven thousand and five hundred cotton accessions. The present investigation was to analyse genetic diversity of tolerant and susceptible accessions in order to select most diverse parents for development of tolerant cotton cultivars against waterlogging. Out of 48 SSR primer pairs surveyed, 38(79%) were showing polymorphism. The identified polymorphic markers detected a total of 104 alleles with mean value of 2.67 alleles/loci. The polymorphism information content (PIC) valued from 0.18 to 0.66 with an average value of 0.43. The major allele frequency ranged from 0.33 to 0.89 with the mean value of 0.57. The observed heterozygosity values for the marker loci varied from 0.00 to 1.00 with the mean value of 0.64. Six SSR loci did not show heterozygosity/any heterozygotes while remaining loci showed the range of values from 0.13 to 1.00. The expected heterozygosity (gene diversity) values ranged from 0.20 to 0.72. Jaccard coefficient based dissimilarity index between the genotypes ranged from 0.27 to 0.63 indicating existence of moderate variation between and within the waterlogging tolerant and susceptible genotypes at the DNA level. Based on the genetic diversity analysis, two tolerant genotypes namely IC563998 and LRA5166 and two susceptible accessions namely IC357607 and IC356708 were identified for utilization in the crossing programme against waterlogging conditions.

Key words : Cotton, genetic diversity, *G. hirsutum*, SSR, waterlogging

Cotton is an important fibre crop for Indian economy and farming community. Due to climate change, biotic and abiotic stress particularly waterlogging affects the cotton ecosystem to a greater extent. Waterlogging is one of the major challenges to cotton cultivation in countries such as India, Pakistan and China (Pang *et al.*, 2004). Waterlogging can cause yield reductions from 10 per cent (Bange *et al.*, 2004) to 40 per cent (Hodgson and Chan, 1982). The effect of waterlogging is much severe where the heavy clay soils (vertisol) with poor drainage rates are present (Bange *et al.*, 2004; Chan and Hodgson, 1981). Since there is no aerenchyma formation, the transport and movement of oxygen to the roots and removal of toxic compounds such as CO^2, methane and ethylene from the roots leads to deleterious effects (Conaty *et al.*, 2008). The oxygen deprivation changes gene expression, physiological and biochemical reactions in the roots and shoots (Meyer *et al.*, 1987; Christianson *et al.*, 2010; Najeeb *et al.*, 2015; Zhang *et al.*, 2017). Water and nutrient uptake, particularly nitrogen uptake in roots are reduced leading to stunted growth and wilting (Bange *et al.*, 2004). Root adaptations like lenticels and adventitious roots have been reported under waterlogged conditions (Hebbar *et al.*, 2001; Hebbar, 2003; Reicosky *et al.*, 1985; Schaefer *et al.*, 1987). Our studies (unpublished observations) showed that for preliminary screening against waterlogging conditions, lenticels and adventitious roots formation are very much important for the identification of tolerant and susceptible accessions. The aim of the study was to characterize tolerant and susceptible cotton (*G. hirsutum*) accessions against waterlogging conditions with Simple Sequence Repeat (SSR) markers.

MATERIALS AND METHODS

Seven thousand and five hundred cotton (*G. hirsutum*) germplasm accessions received from medium term cold storage facility of ICAR-CICR, Nagpur were screened against water logging conditions during the cropping season 2012-2013 and 2013-2014. Plots with closed bunds were created in the Panjari farm of ICAR-CICR, Nagpur to maintain water logged conditions. Forty five days old seedlings were kept under water logged conditions in the plots continuously for 25 days. A total of 150 accessions that were selected from field conditions during 2012-2013 and 2013-2014 were screened against water logging in pots to further identify the most tolerant lines at Panjari farm, Nagpur and Regional station, Coimbatore. Three replications of treatments were maintained with control for the screening program in completely randomised design. Morphological, anatomical, physiological and biochemical characterization were carried out to identify tolerant and susceptible accessions. Adaptations like lenticels and adventitious roots formation was found to be a good index in screening water logging tolerance. LRA5166 was used as tolerant check in the screening programme. Based on pot culture studies, the following tolerant and susceptible accessions were selected for genetic diversity analysis (Table1).

Total genomic DNA was extracted from fresh leaf samples of seedlings of both tolerant and susceptible accessions using a rapid extraction method (Paterson *et al.*, 1993). The DNA quality was assessed through 0.8 per cent agarose gel electrophoresis. All samples were adjusted to a uniform DNA concentration of 10 ng/µl using sterile distilled water. A set of 48 SSR primer pairs designed from the publicly available genome sequences of cotton was used for genotyping and polymorphism assessment. The sequence information of these primers is available at http://www.cottonmarker.org.PCR amplification was performed in a 25 µl reaction volume containing 1X PCR buffer with 2.5 mM MgCl2, 0.2 mM each of dNTPs, 0.4 µM each of forward and reverse primer, 0.5 U Taq polymerase and 2 µl of DNA (10 ng/µl) as Template. Genomic regions pertaining to SSR markers were amplified under the following PCR cycle conditions: 94°C for 4 min for initial denaturation, 35 cycles comprising of three steps *viz.*, denaturation at 94°C for 30 seconds, annealing at 55°C for 30seconds, and extension at 72°C for 30seconds. Reaction was finally ended with incubation at 72° C for 10 min and followed by hold at 4° C using a thermal cycler (Bioradi Cycler). The PCR products were resolved by horizontal electrophoresis system using 3.5 per cent agarose in 1X TBE buffer and polymorphism was visualized by staining with ethidium bromide and documented using a Gel Documentation system (AlphaImager). The

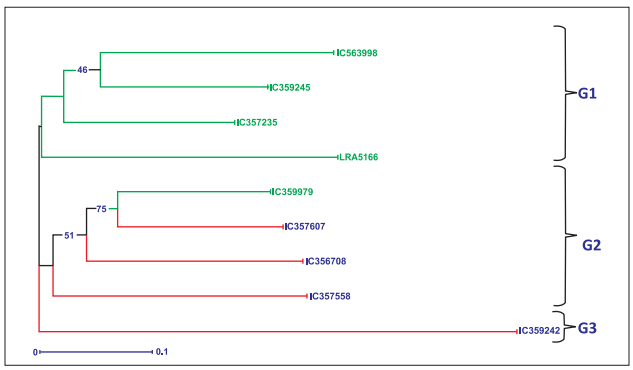

Fig. 1. Dengrogram depicting genetic relationships among tolerant and susceptible accessions of *G. hirsutum* against water logging (Bootstrap values > 40% are shown)

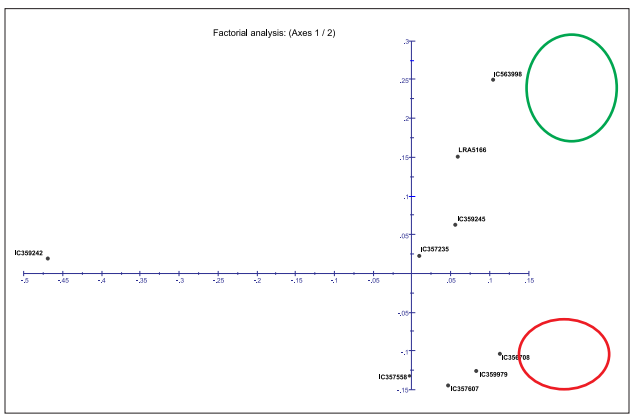

Fig. 2. Factorial analysis (Principal Coordinate Analysis) of tolerant and susceptible accessions of *G. hirsutum*

amplicons were scored across the lanes comparing their respective molecular weights. Presence of a band was scored as "1"and absence of a band as "0" for further analysis.

Level of polymorphism with respect to each marker on allele frequencies, observed heterozygosity (Ho), gene diversity (expected heterozygosity, He) and polymorphism information content (PIC) was estimated using Power Marker version 3.25 (Liu and Muse, 2005). The SSR allelic data was used to calculate the pairwise dissimilarity co-efficients (simple matching) and construct a neighbour joining tree (Perrier *et al.*, 2003) using DAR win (Dissimilarity Analysis and Representation for windows) 6.0.012 (Perrier and Jacquemod Collet, 2006) to understand the relationship among the genotypes, using the Sokal and Michener index. Bootstrap dissimilarity matrices were calculated by drawing 1000 entries. A factorial analysis, Principal coordinates analysis (PCoA) based on the pairwise distance matrix was performed to visualize the overall representation of diversity of tolerant and susceptible accessions.

RESULTS AND DISCUSSION

Waterlogging is one of the major impediments to reach full yield potential in the cotton production systems. Screening of cotton germplasm to identify tolerant/resistant accessions is the first step in the strategies to mitigate the problem of waterlogging. The identified accessions are then used as parent material to introgress the waterlogging-tolerance trait into elite cotton varieties. Based on field and pot culture studies conducted with seven thousand accessions, a total of 150 accessions were identified for their tolerance to water logging conditions. Based on morphological, anatomical, physiological and biochemical characterization, four tolerant (IC 359979, IC359245, IC357235, IC563998) and four susceptible (IC357558, IC359242, IC357607 and IC356708) accessions were selected. Conaty *et al.*, (2008) reported that the most waterlogging tolerant cultivars were Gohar 87, Pima A-8, Sicot 71, Sicot 73 and Sicot 80 which were bred in heavy clays, while the most susceptible lines were Georgia King, LA887,

Table 1. List of accessions used in the genetic diversity analysis

S. No	Name of the accessions	Tolerant/susceptible against water logging conditions	Source/Origin*
1.	IC359979	Tolerant	India
2.	IC359245	Tolerant	USA
3.	IC357235	Tolerant	Tanzania
4.	IC563998	Tolerant	India
5.	LRA5166	Tolerant check	India
6.	IC357558	Susceptible	India
7.	IC359242	Susceptible	USA
8.	IC357607	Susceptible	India
9.	IC356708	Susceptible	USA

(*Source: Catalogue on Cotton Genetic Resources in India, ICAR-CICR, Nagpur, March 2008)

Table 3. Dissimilarility index among the tolerant and susceptible accessions

	IC357558	IC359242	LRA5166	IC563998	IC359245	IC357235	IC359979	IC357607
IC359242	0.58							
LRA5166	0.62	0.40						
IC563998	0.57	0.40	0.40					
IC359245	0.60	0.43	0.34	0.27				
IC357235	0.53	0.36	0.35	0.37	0.36			
IC359979	0.59	0.40	0.43	0.39	0.36	0.28		
IC357607	0.63	0.51	0.45	0.44	0.44	0.39	0.35	
IC356708	0.61	0.46	0.41	0.46	0.47	0.41	0.49	0.43

Table 2. Diversity measures of SSR loci for characterization of tolerant and susceptible accessions against waterlogging conditions

Marker	Major allele frequency	Allele No.	Gene diversity	Heterozygosity	PIC
BNL1721	0.63	2	0.47	0.00	0.36
BNL1408	0.56	4	0.62	0.50	0.57
BNL1434	0.89	2	0.20	0.22	0.18
BNL1694	0.44	3	0.62	1.00	0.54
BNL2544	0.78	2	0.35	0.00	0.29
BNL1035	0.33	4	0.72	0.67	0.66
BNL3545	0.39	4	0.71	1.00	0.66
BNL3806	0.50	3	0.62	1.00	0.54
NAU3961	0.75	2	0.38	0.00	0.30
NAU1167	0.44	3	0.60	1.00	0.52
TMB1660	0.67	2	0.44	0.00	0.35
BNL0852	0.56	2	0.49	0.00	0.37
BNL1414	0.69	3	0.48	0.38	0.43
BNL1672	0.60	2	0.48	0.80	0.36
BNL3408	0.50	3	0.62	1.00	0.54
BNL3426	0.89	2	0.20	0.00	0.18
BNL3254	0.44	5	0.69	0.88	0.64
BNL3371	0.50	3	0.55	1.00	0.45
BNL2986	0.81	3	0.32	0.38	0.29
NAU3401	0.50	3	0.55	1.00	0.46
NAU2152	0.39	3	0.66	1.00	0.59
NAU2162	0.69	2	0.43	0.13	0.34
NAU 1233	0.56	3	0.59	0.63	0.52
NAU 2169	0.79	2	0.34	0.43	0.28
NAU 2439	0.64	3	0.52	0.57	0.46
NAU3433	0.57	3	0.57	0.86	0.50
NAU 2503	0.57	2	0.49	0.86	0.37
NAU 3095	0.71	2	0.41	0.00	0.32
NAU 2190	0.75	3	0.40	0.50	0.35
NAU 1369	0.50	2	0.50	1.00	0.38
TMB 1484	0.50	2	0.50	1.00	0.38
BNL 3563	0.63	2	0.47	0.25	0.36
BNL 3090	0.50	3	0.59	1.00	0.51
BNL 3848	0.38	3	0.66	1.00	0.59
NAU 2419	0.50	3	0.61	1.00	0.54
NAU 3092	0.50	2	0.50	1.00	0.38
NAU 3053	0.39	3	0.66	1.00	0.59
NAU 3442	0.50	2	0.50	1.00	0.38
NAU 1366	0.50	2	0.50	1.00	0.38
Mean	0.57	2.67	0.51	0.64	0.43
Max	0.89	5.00	0.72	1.00	0.66
Min	0.33	2.00	0.20	0.00	0.18

DP16, DP90 and CIM443 which were bred in lighter texture soils. Because of reproducibility, multiallelic nature, codominant inheritance, abundance and wide distribution throughout the genome, microsatellite or simple sequence repeat (SSR) markers are preferred in the genetic diversity studies in crops including cotton (Varshney *et al.*, 2005, Rakshit *et al.*, 2011; Sapkal *et al.*, 2011; Ahmed *et al.*, 2013, Tyagi *et al.*, 2014; Muthusamy *et al.*, 2015; Abd El-Moghny *et al.*, 2017, Santhy *et al.*, 2020). Out of 48 SSR primer pairs surveyed, 38 (79%) showed polymorphism. The identified polymorphic markers detected a total of 104 alleles with mean value of 2.67 alleles/loci (Table 2). The polymorphism information content (PIC) valued

from 0.18 to 0.66with a mean value of 0.43. The major allele frequency ranged from 0.33 to 0.89 with an average value of 0.57. The observed heterozygosity values for the marker loci varied from 0.00 to 1.00 with the mean value of 0.64. Six SSR loci did not show heterozygosity for any heterozygotes, while remaining loci showed the range of values from 0.13 to 1.00. The expected heterozygosity (gene diversity) values ranged from 0.20 to 0.72.The genetic dissimilarity among the nine germplasm accessions including tolerant check LRA5166 (Table 3.) observed to vary from 0.63 (IC357558 with IC357607) to 0.27 (IC359245 with IC563998). Between the accessions of waterlogging tolerant and susceptible group, highest dissimilarity of 62 per cent was observed for LRA5166 and IC357558 followed by 60 per cent for IC357558 and IC359245 while the maximum genetic similarity of 73 per cent was observed between IC563998 and IC359245 followed by IC357235 and IC359979. Among the waterlogging tolerant group, highest dissimilarity of 43 per cent was found between LRA5166 and IC359979 while highest similarity of 73 per cent was observed between IC359245 and IC563998. Among the waterlogging susceptible accessions, maximum dissimilarity of 63 per cent was recorded between IC357558 and IC357607 while minimum of 43 per cent was observed between IC357607 and IC356708. Germplasm accessions in waterlogging susceptible group were observed to be more diverse than the accessions of the tolerant group.

Based on the neighbour joining analysis, 9 germplasm accessions including tolerant check (LRA5166) were grouped into three clusters with varying bootstrap support in the dendrogram using DAR win statistical package (Fig.1). Allthe tolerant accessions except IC359979 were present in the cluster G1 (4 accessions). The susceptible accessions were grouped into two clusters particularly G2 (4 accessions) and G3 (1 accession). Principal Coordinates Analysis (PCoA) was carried out to visualize the overall representation of genetic diversity (Fig. 2). Based on the genetic diversity analysis, two tolerant genotypes namely IC563998 and LRA5166 and two susceptible accessions namely IC357607 and IC356708 were identified for utilization in the crossing programme for further research on waterlogging tolerance.

Due to climate change and global warming, biotic and abiotic stresses are affecting the cotton production ecosystems. Identification and development of genotypes against abiotic stresses particularly waterlogging is of paramount importance in the mitigation strategies. Based on morphological, anatomical, physiological and biochemical analysis, germplasm accessions such as IC359979, IC359245, IC357235, IC563998and IC357558, IC359242, IC357607, IC356708 were identified as tolerant and susceptible respectively. Basedongenetic diversity analysis, two tolerant genotypes namely IC563998 and LRA5166 and two susceptible accessions namely IC357607 and IC356708 were identified for utilization in the crossing programme against waterlogging conditions. The identified accessions will be utilized in the development of varieties through marker assisted selection/backcross (MAS/MAB) breeding programme.

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