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# Screening of cotton (Gossypium hirsutum L.) genotypes for drought tolerance

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#### Abstract

The experiment entitled "screening the cotton (Gossypium hirsutum L.) Genotypes for drought tolerance" was conducted at Department of Biochemistry, Main Cotton Research Station, Navsari Agricultural University, Surat. Twelve genotypes were selected for screening in drought tolerance. Seeds were grown in pots filled with sand culture for 15 days. The seedling was subjected to control and 10 % PEG-6000 treatment for 48 hour and control was taken without PEG treatment. Samples were analyzed for relative water content, membrane stability index, proline, chlorophyll stability index and molecular diversity using molecular markers. Two genotypes GSHV-172 and GISV-272 showed highest relative water content (70.36 and 72.31 %) in PEG treatment and (95.83 and 95.71 %) respectively in control while membrane stability index was found (73.03 and 66.49 %) in GSHV-172 and GISV-272 respectively in PEG treated samples. Proline contend was found higher in same genotype. Two genotypes GSHV-172 and GISV-272 showed highest Chlorophyll stability index compared to other genotypes. So based on biochemical parameters GSHV-172 and GISV-272 found drought tolerant genotype, rest of genotypes were moderately tolerance to drought susceptible. Total twelve RAPD were amplified to generate the 86 fragments and obtained 71 polymorphic bends. The percent polymorphism obtained for RAPD primers were ranked from 25 % to 100 %. The cluster analysis showed the highest similarity 94 % was observed between GISV-272 and GSHV-172 while, the lowest similarity 77 % was observed between Surat dwarf and GSHV-01/13387.

Keywords: Relative water content, Membrane stability index, RAPD

#### Introduction

Cotton is an important industrial crop which in popularly known as "white gold" and the "king of fiber". This commercial crop with unique industrial properties and major source of highquality natural fiber and edible oil, cotton enjoys great demand across the globe. (Wendel et al., 2016) [16] The cotton (Gossypium hirsutum L.), an important fibre crop, is grown throughout India under both rainfed and irrigated conditions on an area of 9.5 million ha (Yang et al., 2014)<sup>[37]</sup>. India has the largest land area under cotton cultivation and is the second largest producer of cotton in the world (Singh, Kairon, 2013) [29]. It includes approximately 50 species distributed worldwide. Among these 50, two diploid (G. arboreum and G. herbaceum) and two tetraploid (G. hirsutum and G. barbadense) species are under cultivation in tropical and sub-tropical environmental conditions. Four species are under commercial cultivation G. herbaceum L. (2n=26), G. arboretum L. (2n=26), G. hirsutum L. (2n=52) and G. barbandense L. (2n=52). Improving drought tolerance is complex for researchers because, under drought, the plant itself adopts various strategies to combat stress depending on the level of water stress, the length of time to which the plant is subjected to water stress and the genotypes of plant species. (Boutree et al, 2010)<sup>[9]</sup> Examination of biochemical characters under stress condition is helpful to know the adaptations mechanism against the harsher environment (Prajapat et al., 2018)<sup>[23]</sup>.

Drought is commonly defined as the absence of adequate moisture for a plant to grow normally and complete its life cycle (Bartels, Sunkar, 2005)<sup>[5]</sup>. Drought is one of the most critical abiotic stresses that limit crop growth and productivity worldwide. Drought is considered a multidimensional stress that leads to changes in the physiological, morphological, ecological, biochemical and molecular characteristics of plants. (Hasan *et al.*, 2018)<sup>[15]</sup>. The symptoms of drought stress also vary with the plant species, developmental stages, growth conditions, and environmental factors (Arbona *et al.*, 2013, Bhargava *et al.*, 2013)<sup>[4,7]</sup>.

Drought stress inhibits plant growth and development (Wang et al., 2003)<sup>[35]</sup> but enables root length proliferation to acquire water from the deep soil and tolerate the stress (Hufstetler et al., 2007, Afshari, et al., 2011)<sup>[17, 2]</sup>. The root/shoot ratio also increases, indicating water acclimatization and enhanced tolerance (Kumar et al. 2010, Sumartini et al., 2013)<sup>[19, 31]</sup>. Decreased shoot length is observed due to the blockage of vascular tissue vessels and a reduction in cell elongation (Abdalla et al., 2007)<sup>[1]</sup>. Generally, drought symptoms are mostly observed in the leaves of plants showing loss of turgor, drooping, wilting, etiolation, yellowing, and premature downfall (Akhtar, Nazir., 2013, Sapeta et al., 2013)<sup>[3, 27]</sup>. The photosynthetic rate was found to decrease under drought conditions in different plant species (Chen et al., 2010)<sup>[10]</sup>. Plants grown under drought conditions have lower stomatal conductance, reduced CO<sub>2</sub> fixation, and decreased photosynthesis, which result in reduced growth and yield of plants. Severe drought stress also inhibits the photosynthesis of plants by causing changes in the chlorophyll content and damaging the photosynthetic apparatus (Dalton et al., 1998) <sup>[11]</sup>. Drought is an abiotic stress, it has drastic effect on plant growth and crop productivity (Quisenberry et al., 1985)<sup>[24]</sup>.

The entire cotton plant has the potential to be a source of valuable compounds, such as terpenes, phenolics, fatty acids, lipids, carbohydrates, and proteins (Shakhidoyatov *et al.*, 1997, Perveen, *et al.*, 2001) <sup>[28, 22]</sup>. These compounds, which are distributed in seeds, bolls, calyx, leaves, stalks, stems, and roots of the plant (Hu *et al.*, 2011, Haleem *et al.*, 2014) <sup>[16, 14]</sup> play functional biological roles in humans and animals (Essien *et al.*, 2011, Sánchez-Muñoz *et al.*, 2012, Rogerio *et al.*, 2009) <sup>[13, 26, 25]</sup>.

#### **Material and Methods**

The investigation on "screening the cotton (*Gossypium hirsutum* L.) Genotypes for drought tolerance" was carried out at the Department of Biochemistry, Main Cotton Research Station, Navsari Agricultural University, Surat. The statistical design used for the study was completely randomized design. The experiment was carried out with twelve cotton genotypes using CRD design.

**Geographical features:** Geographically, Surat is located at a cross point of  $20^{\circ}12$ ' N latitude and  $72^{\circ}52$ ' E longitudes with an altitude of near at 12 (11.34) meters above the mean sea level in South of Gujarat. The place is located at near Science Center, Surat Municipal Corporation, Athwa lines, Surat.

The 12 cotton genotypes seeds were grown for 15 days in sand culture with 10 plants for each genotype. Four kg of soil, peat and sand in the ratio 1:1:1 was filled in 5 kg capacity pots during *Kharif* (June- September 2020). After 15 days, the

plants were uprooted and dipped in 10 % PEG-6000 for 48 hours. After 48 hour the leaf samples were taken out and used for physiological and biochemical parameters from control and PEG treated condition. Molecular analysis was done from 15 days old genotypes.

V1 – G Cot 10	V5 - GSHV-180	V9 - BC-68-2
V2 - G.Cot.100	V6 - G.Cot.16	V10 - American nectariless
V3 -GISV- 272	V7 - LRA-5166	V11 - Surat dwarf
V4 - GSHV-172	V8 - G.N.Cot.22	V12 - GSHV-01/13387

ANOVA was carried out to test difference in treatment using completely randomized design with three repetitions. Data were analyzed using OPSTAT (O.P. Sheoran Programmer, Computer Section, CCS HAU, Hisar) statistic software. The critical difference (CD) among the variances was calculated at  $p \leq 0.05$ . The molecular data were analyzed using unweight pair group method using arithmetic average (UPGMA) method by NTSYS-pc version 2.02.

Table 2: Observation recorded

Sr. No	Parameter	Reference
1	Relative water content (%)	Turner (1986) <sup>[33]</sup>
2	Membrane stability index (%)	Martineau et al., (1979) <sup>[20]</sup>
3	Proline ( $\mu g g^{-1}$ )	Bates et al. (1973) <sup>[6]</sup> .
4	Chlorophyll Stability (%)	Sibasubramanian (1992) <sup>[30]</sup>
5	Molecular Diversity using	Botstein et al., (1980) <sup>[8]</sup> and
5	molecular marker	Doyle and Doyle (1987) <sup>[12]</sup>

#### **Results and Discussion**

The data presented in Table 3 indicate that due to drought stress, there was decline in normal condition. The Relative water content, MSI percentage, Proline and Chlorophyll stability index was recorded at 15 days after sowing from control and 48-hour PEG Treated condition. Genotype GSHV-172 and GISV-272 showed significantly higher among all genotypes under control and PEG treated condition. Techawongstin et al. (1993) [32] reported a similar phenomenon in water-stressed hot pepper. Results in total dysfunction and it is generally accepted that the maintenance of integrity and stability of membranes under drought stress is a major component of drought tolerance in plants (Vaidya et al. 2015) <sup>[34]</sup>. Iqbal et al. (2016) <sup>[18]</sup> who reported that, the accumulation of proline in drought tolerant and drought susceptible cultivars has revealed the significance of this osmolyte. Proline content has been shown to accumulate upon desiccation in leaves of many plant species. It has been suggested by Jones et al. 1980. The Same result was revealed by the Patil et al. (2011)<sup>[21]</sup> as Chlorophyll stability index.

 Table 3: Relative water content (%), Membrane stability index (%) Proline (µg g<sup>-1</sup>) Chlorophyll stability index (%) of different genotypes under control and PEG treated condition

		Relative wate	er content (%)	Membrane stab	ility index (%)	Proli	ne (µg g <sup>-1</sup> )	Chlonophyll	
Sr. No	Genotypes	Control	PEG treated	Control	PEG treated	Control	PEG treated	stability index (%)	
		condition	condition	condition	Condition	condition	condition	stability muex (76)	
$V_1$	G. Cot.10	89.48	49.52	62.90	51.56	0.50	1.28	65.81	
$V_2$	G. Cot.100	93.10	51.18	66.77	58.06	0.73	3.32	71.36	
<b>V</b> <sub>3</sub>	GISV-272	95.71	72.31	91.07	66.49	0.86	4.18	83.43	
$V_4$	GSHV-172	95.83	70.36	92.07	73.03	0.91	4.06	86.76	
$V_5$	GSHV-180	94.02	52.80	83.36	52.75	0.62	2.44	79.98	
$V_6$	G. Cot.16	92.54	53.79	87.79	62.93	0.67	3.76	80.28	
<b>V</b> <sub>7</sub>	LRA-5166	95.04	53.66	81.48	52.71	0.62	3.86	76.34	
$V_8$	G.N. Cot.22	95.20	62.57	89.40	53.49	0.65	1.98	74.96	
$V_9$	BC-68-2	90.34	49.80	54.09	50.62	0.61	1.29	65.31	

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V <sub>10</sub>	American nectariless	90.96	58.01	75.43	51.16	0.70	2.30	80.56
V <sub>11</sub>	Surat dwarf	92.54	64.89	77.23	57.26	0.69	2.09	78.34
V <sub>12</sub>	GSHV-01/13387	94.59	53.05	77.44	54.98	0.69	2.98	79.88
S.E.M		0.96	0.60	0.55	0.33	0.01	0.03	1.471
CD at $p \le 0.05$		2.83	1.76	1.60	0.97	0.04	0.08	0.708
CV%		1.79	1.80	1.21	1.01	3.50	1.66	1.128

### Polymorphism as detected by RAPD analysis

The Table 4 percent polymorphism obtained for RAPD primer were ranged from 25 % to 100% with an average value of 82.55 % per primer. The polymorphism information content (PIC) values for RAPD marker from 0.08-0.50. The performance of individual primer to amplify genomic DNA of

12 cotton genotypes is discussed as under.

**OPB-07:** Out of which 7 fragments were polymorphic and 1 fragment were monomorphic having 87.50 % polymorphism and PIC value 0.47

OPM-07: Out of which 3 fragments were polymorphic and 0 fragment were monomorphic having 100.00 % polymorphism and PIC value 0.50

		Relative water content (%)		Membrane stab	ility index (%)	Prolin	Chlorophyll	
Sr. No.	Genotypes	Control	PEG treated	Control	PEG treated	Control	PEG treated	stability
		condition	condition	condition	Condition	condition	condition	index (%)
$V_1$	G. Cot.10	89.48	49.52	62.90	51.56	0.50	1.28	65.81
$V_2$	G. Cot.100	93.10	51.18	66.77	58.06	0.73	3.32	71.36
<b>V</b> <sub>3</sub>	GISV-272	95.71	72.31	91.07	66.49	0.86	4.18	83.43
$V_4$	GSHV-172	95.83	70.36	92.07	73.03	0.91	4.06	86.76
V5	GSHV-180	94.02	52.80	83.36	52.75	0.62	2.44	79.98
$V_6$	G. Cot.16	92.54	53.79	87.79	62.93	0.67	3.76	80.28
$V_7$	LRA-5166	95.04	53.66	81.48	52.71	0.62	3.86	76.34
$V_8$	G.N. Cot.22	95.20	62.57	89.40	53.49	0.65	1.98	74.96
V9	BC-68-2	90.34	49.80	54.09	50.62	0.61	1.29	65.31
$V_{10}$	American nectariless	90.96	58.01	75.43	51.16	0.70	2.30	80.56
V <sub>11</sub>	Surat dwarf	92.54	64.89	77.23	57.26	0.69	2.09	78.34
V12	GSHV-01/13387	94.59	53.05	77.44	54.98	0.69	2.98	79.88
SEm		0.96	0.60	0.55	0.33	0.01	0.03	1.471
CD at $p \le 0.05$		2.83	1.76	1.60	0.97	0.04	0.08	0.708
CV %		1.79	1.80	1.21	1.01	3.50	1.66	1.128

**OPM-13:** Out of which 7 fragments were polymorphic and 0 fragment were monomorphic having 100.00 % polymorphism and PIC value 0.41.

**OPM-19:** Out of which 1 fragment were polymorphic and 3 fragments were monomorphic having 25.00 % polymorphism and PIC value 0.08

**OPM-20:** Out of which 9 fragments were polymorphic and 0 fragments were monomorphic having 100.00% polymorphism and PIC value 0.50.

**OPX-13:** Out of which 3 fragments were polymorphic and 5 fragments were monomorphic having 37.50 % polymorphism and PIC value 0.19.

**OPC-11**: Out of which 4 fragments were polymorphic and 4 fragments were monomorphic having 50.00 % polymorphism and PIC value 0.41.

**OPJ-05:** Out of which 10 fragments were polymorphic and 0 fragments were monomorphic having 100.00 % polymorphism and PIC value 0.50.

**OPJ-19:** Out of which 9 fragments were polymorphic and 0 fragments were monomorphic having 100.00 % polymorphism and PIC value 0.44.

**OPA-01:** Out of which 4 fragments were polymorphic and 0 fragments were monomorphic having 100.00 % polymorphism and PIC value 0.50.

**OPC-20:** Out of which 11 fragments were polymorphic and 0 fragments were monomorphic having 100.00 % polymorphism and PIC value 0.47.

**OPC-07:** Out of which 3 fragments were polymorphic and 2 fragments were monomorphic having 60.00 % polymorphism and PIC value 0.15.

Sr. No	Name of primers	Total number of bands	Number of Monomorphic Bands	Number of Polymorphic Bands	Percent Polymorphism	PIC Value
1	OPB-07	8	1	7	87.50	0.47
2	OPM-07	3	0	3	100.00	0.50
3	OPM-13	7	0	7	100.00	0.41
4	OPM-19	4	3	1	25.00	0.08
5	OPM-20	9	0	9	100.00	0.50
6	OPX-13	8	5	3	37.50	0.19
7	OPC-11	8	4	4	50.00	0.41
8	OPJ-05	10	0	10	100.00	0.50

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9	OPJ-19	9	0	9	100.00	0.44
10	OPA-01	4	0	4	100.00	0.50
11	OPC-20	11	0	11	100.00	0.47
12	OPC-07	5	2	3	60.00	0.15
		86	15	71	82.55	4.62

Table 5: Genetic similarity coefficient between the cotton genotypes based on the RAPD data

Genotypes	G Cot 10	G Cot 100	<b>GISV-272</b>	GSHV-172	<b>GSHV-180</b>	G Cot 16	LRA-5166	GN Cot 22	BC-68-2	AN	SD	GSHV-01/13387
G. Cot.10	1.00											
G. Cot.100	0.85	1.00										
GISV-272	0.83	0.87	1.00									
GSHV-172	0.78	0.83	0.94	1.00								
GSHV-180	0.77	0.84	0.84	0.81	1.00							
G. Cot.16	0.74	0.79	0.85	0.81	0.84	1.00						
LRA-5166	0.81	0.82	0.81	0.77	0.79	0.81	1.00					
G.N. Cot.22	0.61	0.57	0.54	0.51	0.56	0.60	0.67	1.00				
BC-68-2	0.70	0.70	0.64	0.61	0.71	0.76	0.79	0.69	1.00			
AN	0.83	0.83	0.88	0.83	0.78	0.85	0.79	0.51	0.70	1.00		
SD	0.67	0.65	0.65	0.63	0.55	0.63	0.63	0.60	0.69	0.65	1.00	
GSHV-01/13387	0.67	0.63	0.61	0.61	0.50	0.50	0.63	0.57	0.56	0.60	0.77	1.00



Fig 1: Dendrogram depicting the genetic relationship among 12 cotton genotypes based on RAPD data

#### Genetic similarity

Genetic similarity was determined under Table 5 for each pair of 12 populations which revealed that the genetic similarity was minimum 0.50 and maximum 0.94.

#### Conclusion

Based on biochemical observation among twelve cotton genotypes, Genotypes GSHV-172 and GISV-272 showed highest relative water content, membrane stability index, and proline and chlorophyll stability index compared to other genotypes. Eighty-six fragment and seventy one polymorphic bands were obtained using 12 RAPD primers. The cluster analysis showed the highest similarity 94% between GISV-272 and GSHV-172 belong to same cluster. From biochemical observation genotypes GSHV-172 and GISV-272 were found drought tolerant which having similar cluster in Dendrogram and highest genetic similarity.

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