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RESEARCH PAPER

Screening *hirsutum* cotton genotypes for water stress

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Abstract: Performance of twelve *hirsutum* cotton genotypes for water stress was evaluated for two years (*kharif* 2015-16 and 2016-17) under field experimentation at Agriculture Research Station, Dharwad farm. Morpho-physiological and yield parameters were recorded in all the genotypes grown under water stressed and nonstressed conditions. The per cent reduction in various parameters *viz.*, relative water content, excised leaf water loss, cell membrane stability, specific leaf weight, chlorophyll content, stomatal frequency, trichomes density and yield and yield components of each genotype in stressed over nonstressed condition was worked out. Based on the criteria of least per cent reduction in maximum number of parameters, five cotton genotypes *viz.,* GSHV-169, BS-37, BS-39, AKH-09-5, ARBH-1352 were identified as more tolerant to water stress.

Key words: Cotton, Stomatal frequency, Trichome density, Water stress

Introduction

Cotton is one of the most important economic fibre crops in the world. Cotton is known as the 'King of fibre' and also 'White Gold' is most vital crop of commerce to many countries including India. Cotton is cultivated in 70 countries of the world with the total coverage of 331 lakh ha, production of 1166 lakh bales and a productivity of 766 kg lint per ha. India being the traditional home for cotton and cotton textiles, the cultivated area occupying about 118.81 lakh ha producing 352 lakh bales with the productivity of 504 kg lint per ha. In Karnataka, it is grown in an area of 6.12 lakh ha with a production of 20 lakh bales and productivity of 556 kg lint per ha (Anon., 2016). Though, India has the largest area under cotton, it ranks third in production due to low productivity. The major reasons for low yield in India are biotic, abiotic, and technological problems. One of the major abiotic stresses affecting plant productivity is water stress resulting through drought which limits crop growth and productivity.

Water availability and quality affect the growth and physiological processes of all plants since water is the primary component of actively growing plants ranging from 70-90% of plant fresh mass. Due to its predominant role in plant nutrient transport, chemical and enzymatic reactions, cell expansion and transpiration, water stresses result in anatomical and morphological alterations as well as changes in physiological and biochemical processes affecting functions of the plants. Plant water deficits depend both on the supply of water to the soil and the evaporative demand of the atmosphere. In general, plant water stress is defined as the condition where a plant's water potential and turgor are decreased enough to inhibit normal plant function. The effects of water stress depend on the severity and duration of the stress, the growth stage at which stress is imposed and the genotype of the plant.

Cotton is one of the most important economic crops in world. It is grown in both dry land and irrigated areas. The effect of water stress on growth, yield components and quality characters of cotton are widely different. The turgor loss in the tissue is the first effect of water stress that influences cell growth rate and its final effect on yield. Among the abiotic stresses, drought is recognized as the most devastating cause which limits the fiber yield and lint quality in cotton production. The flowering and boll development stages are the critical stages of water requirement that determine the final yield in cotton. Short-duration water stress occurring during these stages significantly affects various physiological and biochemical characters such as leaf expansion, photosynthesis, carbon and antioxidant metabolism.

Various methodologies have been employed to identify drought tolerant genotypes. Researchers consider physiological parameters to compare the change in seed cotton yield between stressed and non stressed conditions. Yield loss is the major concern to farmers and scientists. Hence, emphasis is given on selection genotypes on yield performance under moisture stress conditions. But variation in yield potential arises from factors related to adaptation rather than drought tolerance. Thus, these parameters provide a measure of drought tolerance based on yield loss under drought conditions compared to normal conditions and are being used in screening drought tolerant genotypes (Mitra, 2001).

This study was carried out in order to evaluate *hirsutum* cotton genotypes to drought stress, so that suitable varieties can be recommended for cultivation in drought prone areas.

Material and methods

The two year field experiments were carried out to evaluate hirsutum cotton genotypes to drought stress in Agriculture

Research Station, Dharwad during the *kharif* season 2015 and 2016. Tweleve cotton (*Gossypium hirsutum*) genotypes were grown in split-plot design with two water regimes (rainfed and need based irrigation at flowering and boll development) as the main plot treatment with three replications. The genotypes used were GBHV-182, PH-1060, GSHV-169, TCH-1777, SCS-1062, AKH-09-5, ARBH-1352, BS-37, BS-39, GJHV-516, LRA-5166 (National check), RAH-100 (Local check).

Observation on various parameters was recorded by standard procedures as indicated below.

Specific leaf weight (mg cm^{-2}): Specific leaf weight (SLW) was determined by method as suggested by Radford (1967).

$$SLW = \frac{\text{Leaf dry weight (mg)}}{\text{Leaf area (cm}^{-2})}$$

Relative water content (%): Relative water content (RWC) was estimated by the formula given by Kramer (1983).

$$RWC\,(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Excised leaf water loss (ELWL): Three fully developed leaves were excised and weigh immediately (fresh weight) and samples were left on laboratory benches for six hours. After six hours the weight of wilted leaves was recorded and samples were then dried in oven at 70 °C. The ELWL was calculated using the following formula (Muhammad *et al.*, 2011).

$$ELWL = \frac{\text{Fresh weight} - \text{Wilted weight}}{\text{Dry weight}}$$

Cell membrane stability (CMS): The leaf discs were suspended separately in equal volume of distilled water and subjected to different temperatures (room temperature, 60 and 100 °C) for 20 minutes and electrical conductivity of suspended water (EC_a), 30 minutes (EC_b) and 10 min (EC_c), respectively were recorded (Sullivan, 1971).

$$\text{Per cent leakage} = \frac{EC_b - EC_a}{EC_c} \times 100$$

Stomatal frequency: The stomatal frequency was recorded by following microscopic observation by xylene-thermocole solution impression with scope image 9.0 versions with 10 X magnification per unit leaf area (Brewer and Smith 1997 and Neill, *et al.,* 1990).

Trichome density: The trichome density was counted with the aid of high magnifying power image analyzer (scopeimage 9.0 version) under 10 X magnification. Based on the appearance of the number of trichomes in 10000 µm^2, was expressed in numbers.

Chlorophyll estimation by SPAD (SCMR): SPAD Chlorophyll meter readings measure the greenness or relative chlorophyll content of the leaves

Chlorophyll estimation by DMSO method: Chlorophyll content in the leaves of different hirsutum genotypes of cotton was determined by using dimethyl sulfoxide (DMSO) as given by Shoaf and Loum (1976).

Yield components: Total number of bolls picked from five tagged plants were counted and weighed. Seed cotton was separated and weighed and average was expressed as number of bolls per plant and seed cotton yield (g plant^{-1}). The average weight of randomly selected 20 bolls from the net plot was noted as single boll weight (g boll^{-1}). Total seed cotton yield of all the pickings from net plot area was added, averaged and expressed as seed cotton yield (g plot^{-1}) and was computed to kilogram per hectare (kg ha^{-1}).

The data from two years for all the parameters was pooled and analyzed statistically as prescribed by Gomez and Gomez (1984).

Results and discussion

The physiological parameters of cotton genotypes get affected under water stress condition. The physiological parameters such as chlorophyll, SPAD, excised leaf water loss, specific leaf weight, relative water content, cell membrane stability, stomatal frequency and trichome density are the most important parameters for water stress. For this reason these physiological parameters provides an important clue to the response of plant to water stress (Jamal *et al.,* 2014). Significant reduction in all physiological parameters sunder water stress condition compared to irrigated condition (control), all genotypes responded effectively to water stress.

Less percentage reduction in SLW, CMS and RWC was recorded in GSHV-169(-7.07, 35.39 and 12.45, respectively) followed by BS-37 (-5.56, 16.34 and 5.29, respectively), LRA-5166 (-5.15, 19.13 and 12.39, respectively), ARBH-1352 (-4.89, 14.49 and 6.16, respectively) and BS-39 (-4.86, 14.60 and 7.04, respectively) indicates their higher ability to tolerance to water stress than other genotypes and higher per cent reduction in TCH-1777 (-2.27, 10.88 and 9.80, respectively) followed by AKH-09-5 (-3.53, 10.96 and 14.83, respectively), SCS-1062 (-3.65, 11.62 and 11.06, respectively), RAH-100 (-3.71, 14.94 and 7.34, respectively) and GJHV-516 (-3.76, 13.45 and 7.55, respectively) indicates their susceptibility to water stress condition (Table 1).The present findings are in close agreement with Siddique *et al.* (2001) who reported that tolerant genotypes under rainfed condition showed increased specific leaf weight compared to irrigated condition. The SLW indicates the leaf thickness. Higher the specific leaf weight higher is the number of cells per plant volume and compactness of the cells. SLW under moisture stress condition increases its water use efficiency and also increases the photosynthetic rate.

Relative water content (RWC) is considered as a measure of plant water status, reflecting the metabolic activity in tissues

Table 1. Effect of water stress on specific leaf weight (SLW), excised leaf water loss (ELWL), cell membrane stability (CMS) and relative water content (RWC)

Genotypes	SLW(mg cm^{-2})				ELWL				CMS(%)				Relative water content(%)			
	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction
GBHV-182	6.67	6.97	6.82	-4.50	1.82	1.53	1.68	15.93	65.81	57.75	61.78	12.25	83.95	76.29	80.12	9.12
PH-1060	6.70	7.00	6.85	-4.48	1.88	1.58	1.73	15.96	67.68	57.22	62.45	15.46	83.53	73.65	78.59	11.83
GSHV-169	6.22	6.66	6.44	-7.07	1.40	1.11	1.26	20.71	49.48	31.97	40.72	35.39	83.48	73.09	78.28	12.45
TCH-1777	6.62	6.77	6.69	-2.27	1.70	1.32	1.51	22.35	58.73	52.34	55.54	10.88	83.79	75.58	79.68	9.80
SCS-1062	6.57	6.81	6.69	-3.65	1.54	1.25	1.40	18.83	58.11	51.36	54.74	11.62	80.29	71.41	75.85	11.06
AKH-09-5	6.52	6.75	6.63	-3.53	1.49	1.20	1.34	19.46	56.41	50.23	53.32	10.96	80.64	68.68	74.66	14.83
ARBH-1352	6.75	7.08	6.91	-4.89	1.90	1.67	1.79	12.11	68.72	58.76	63.74	14.49	86.39	81.07	83.73	6.16
BS-37	6.84	7.22	7.03	-5.56	2.15	1.85	2.00	13.95	76.38	63.90	70.14	16.34	84.08	79.63	81.85	5.29
BS-39	6.79	7.12	6.95	-4.86	1.90	1.64	1.77	13.68	69.60	59.44	64.52	14.60	84.35	78.41	81.38	7.04
GJHV-516	6.65	6.90	6.78	-3.76	1.78	1.49	1.64	16.29	64.32	55.67	59.99	13.45	86.12	79.62	82.87	7.55
LRA-5166 (NC)	6.80	7.15	6.97	-5.15	2.00	1.75	1.87	12.50	84.16	68.06	76.11	19.13	87.7	76.83	82.26	12.39
RAH-100 (ZC)	6.47	6.71	6.59	-3.71	1.52	1.22	1.37	19.74	55.70	47.38	51.54	14.94	81.45	75.47	78.46	7.34
Mean	6.63	6.93	6.78	-4.52	1.76	1.47	1.61	16.48	64.59	54.51	59.55	15.61	83.81	75.81	79.81	9.55
	S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %	
WR	0.04		0.22		0.03		0.15		1.02		6.21		0.81		4.92	
G	0.03		0.08		0.01		0.02		0.64		1.82		0.78		2.23	
WR x G	0.05		0.15		0.03		0.08		1.37		3.90		1.35		3.86	

IR – Irrigated RF – Rainfed WR – water regimes G – Genotype NC – National check ZC – zonal check

Table 2. Effect of water stress on total chlorophyll, SPAD values, stomatal frequency and trichome density

Genotypes	Total chlorophyll(mg g fr. wt.$^{-1}$)				SPAD values				Stomatal frequency (number of stomata per 50000 μm^2)				Trichome density (number of trichomes per 10000 μm^2)			
	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction
GBHV-182	2.18	2.05	2.11	5.96	42.09	41.00	41.54	2.59	31.65	26.15	28.90	17.38	21.25	27.00	24.13	-27.06
PH-1060	2.19	2.09	2.14	4.57	42.84	41.11	41.97	4.04	30.20	25.50	27.85	15.56	26.10	28.05	27.08	-7.47
GSHV-169	1.7	1.52	1.61	10.59	39.87	38.54	39.20	3.34	27.45	22.95	25.20	16.39	15.00	23.50	19.25	-56.67
TCH-1777	2.06	1.96	2.01	4.85	41.44	39.76	40.60	4.05	28.15	23.75	25.95	15.63	19.00	30.85	24.93	-62.37
SCS-1062	1.95	1.81	1.88	7.18	41.29	39.26	40.27	4.92	28.35	24.05	26.20	15.17	20.80	25.90	23.35	-24.52
AKH-09-5	1.89	1.74	1.81	7.94	41.15	39.00	40.07	5.22	28.45	24.30	26.38	14.59	25.80	32.50	29.15	-25.97
ARBH-1352	2.23	2.12	2.17	4.93	43.52	41.99	42.75	3.52	32.05	26.35	29.20	17.78	26.45	28.50	27.48	-7.75
BS-37	2.92	2.47	2.69	15.41	44.85	43.04	43.94	4.04	32.65	27.20	29.93	16.69	22.55	25.70	24.13	-13.97
BS-39	2.26	2.14	2.2	5.31	43.52	42.08	42.80	3.31	34.50	26.75	30.63	22.46	27.10	32.30	29.70	-19.19
GJHV-516	2.12	2	2.06	5.66	41.92	40.57	41.24	3.22	29.70	25.35	27.53	14.65	21.25	36.85	29.05	-73.41
LRA-5166 (NC)	2.48	2.29	2.39	7.66	43.79	42.12	42.95	3.81	34.50	28.35	31.43	17.83	19.80	31.65	25.73	-59.85
RAH-100 (ZC)	2.01	1.79	1.9	10.95	40.85	38.80	39.82	5.02	28.35	24.15	26.25	14.81	23.30	25.90	24.60	-11.16
Mean	2.16	2.00	2.08	7.41	42.26	40.60	41.43	3.93	30.50	25.40	27.95	16.72	22.37	29.06	25.71	-29.91
	S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %	
WR	0.01		0.07		0.20		1.22		0.45		2.75		0.64		3.91	
G	0.02		0.05		0.09		0.26		0.35		0.99		0.42		1.20	
WR x G	0.03		0.08		0.24		0.70		0.67		1.90		0.88		2.50	

IR – Irrigated RF – Rainfed WR – water regimes G – Genotype NC – National check ZC – zonal check and used as a most meaningful physiological parameter for dehydration tolerance. Drought tolerant genotype increases the water use efficiency by reducing the water loss by the plant. However, in the events where plant growth was hindered to a greater extent, water use efficiency was also reduced significantly. The cell membrane stability is one of the important screening parameters for water stress condition and it has been measured as percentage injury of leaf tissue (Rashid *et al.,* 2015). The genotypes under irrigated condition were maintained high cell membrane stability than rainfed condition. This drought stress cause cell membrane to lose selective permeability, cellular integrity and capacity for retention of inter cellular substances, ion leakage, decrease in RWC and this may accelerate senescence process (Lukatkin, 2003).

The ELWL attribute to better retention of water content at boll development stage than flowering due to full development of leaves and also presence of drought tolerant character. Higher ELWL content was found under irrigated than rainfed condition (1.76 and 1.47, respectively). Among the genotypes BS-37 (13.95), BS-39 (13.68), LRA-5166 (12.50), ARBH-1352 (12.10) and GBHV-182 (15.93) were maintained lower per cent of ELWL. Hence, these genotypes were considered as tolerant under water stress condition (Table 1).

In water stress condition, RAH-100 (14.81 and -11.16) followed by GJHV-516 (14.65 and -73.41), AKH-09-5 (14.59 and -25.97), SCS-1062 (15.17 and -24.52) and PH-1060 (15.56 and -7.47) recorded least stomatal frequency and trichome density, respectively (table 2). Whereas, in BS-39 (22.46 and -19.19), the per cent reduction was highest followed by LRA-5166 (17.83 and -59.85), ARBH-1352 (17.78 and -7.75) and GBHV-182 (17.38 and -27.06). Water stress induced deformation of trachids in the xylem due to decrease in osmotic potential, the reduction of mitotic activity of mesophyll cells and increase trichome production as well as the decrease in cell size and number of stomata per leaf resulting from water stress (Guerfel *et al.,* 2009). Drought stress results in increased stomatal density in sorghum (McCree and Davis, 1974) and in wheat (Zhang *et al.,* 2006). Drought stress decreases stomatal size results in stomatal morphological changes which could increases the plant adaption to drought stress conditions (Martinez *et al.,* 2007).

Water stress imposed a total chlorophyll and SPAD content (Table 2) over control (7.41 and 3.93 %) respectively. Significantly less per cent reduction was observed in PH-1060 (4.57 and 4.04) followed by TCH-1777 (4.85 and 4.05), ARBH-1352 (4.93 and 3.52) and BS-39 (5.31 and 3.31) over control and the higher per cent reduction was estimated in GSHV-169 (10.59 and 3.34), BS-37 (15.41 and 4.04), SCS1062 (7.18 and 4.92) and AKH-09-5 (7.94 and 5.22), respectively. The significant reduction in chlorphyll and SPAD value under water stress could be related to photo-oxidation due to oxidative stress which reduces photosynthetic process (Hamayun *et al.,* 2010).

Table 3. Effect of water stress on yield and yield components

Genotypes	Number of bolls per plant				Single boll weight($g\ boll^{-1}$)				Seed cotton yield per plant($g\ plant^{-1}$)				Seed cotton yield($kg\ ha^{-1}$)			
	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction	IR	RF	Mean	% reduction
GBHV-182	19.32	17.39	18.35	9.99	4.73	4.34	4.53	8.25	67.67	56.39	62.03	16.67	1461	1347	1404	7.80
PH-1060	17.45	17.51	17.48	-0.34	4.49	4.38	4.43	2.45	63.07	57.01	60.04	9.61	1436	1360	1398	5.29
GSHV-169	11.02	11.27	11.14	-2.27	3.71	3.68	3.7	0.81	33.4	40.45	36.92	-21.11	742	899	820	-21.16
TCH-1777	14.93	15.98	15.46	-7.03	4.19	4.26	4.23	-1.67	52.04	53.15	52.59	-2.13	1160	1206	1183	-3.97
SCS-1062	15.01	15.92	15.46	-6.06	4.28	4.18	4.23	2.34	52.8	52.35	52.57	0.85	1160	1183	1172	-1.98
AKH-09-5	13.49	14.65	14.07	-8.60	4.13	4.07	4.1	1.45	45.72	50.48	48.1	-10.41	1037	1123	1080	-8.29
ARBH-1352	17.32	17.81	17.57	-2.83	4.5	4.45	4.47	1.11	63.22	59.79	61.5	5.43	1422	1405	1413	1.20
BS-37	17.47	19.25	18.36	-10.19	4.56	4.55	4.55	0.22	64.37	68.4	66.38	-6.26	1395	1529	1462	-9.61
BS-39	17.12	18.2	17.66	-6.31	4.56	4.51	4.53	1.10	63.65	63.27	63.46	0.60	1439	1430	1435	0.63
GJHV-516	17.48	16.47	16.97	5.78	4.46	4.31	4.38	3.36	61.66	55.2	58.43	10.48	1391	1315	1353	5.46
LRA-5166 (NC)	18.61	19.32	18.96	-3.82	4.7	4.56	4.63	2.98	67.61	71.98	69.79	-6.46	1468	1513	1491	-3.07
RAH-100 (ZC)	12.85	14.1	13.47	-9.73	3.95	4.01	3.98	-1.52	37.18	49.11	43.15	-32.09	1021	1089	1055	-6.66
Mean	16.00	16.49	16.25	-3.06	4.35	4.27	4.31	1.84	56.03	56.46	56.25		1261	1283	1272	-1.74
	S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %		S.Em±		C.D. @ 5 %	
WR	0.05		0.28		0.01		0.07		0.36		2.20		3		19	
G	0.32		0.92		0.04		0.10		1.25		3.57		24		71	
WR x G	0.44		1.25		0.05		0.15		1.74		4.96		33		96	

IR – Irrigated RF – Rainfed WR – water regimes G – Genotype NC – National check ZC – zonal check

Water stress at peak flowering and boll development stages had detrimental effect on seed cotton yield. Increased moisture stress leads significant decrease in seed cotton yield per plant and reached its maximum under normal irrigation (Hamoda, 2012). Significant reduction in yield and yield attributes under irrigated condition as compared to rainfed condition in all the genotypes was due to the off seasonal heavy rain during the month of September and October which coincides with the boll development stage, which resulted in more number of days to maturity. It also lead to increase in vegetative growth and translocation of photo-assimilates to sink region by increasing total dry matter. Boll development is one of the critical stages in cotton and heavy rainfall at this stage along with irrigation leads to decrease in per cent of boll set and boll weight per plant under irrigated condition. Contrary to this, the rainfed condition received only a rainfall with no irrigation leads to increased seed cotton yield. The more per cent reduction observed in yield and yield parameter in irrigated condition than rain fed condition was due to more number of bolls (16.49), mean single boll weight (4.27 g boll^{-1}) and seed cotton yield per plant (56.46 g plant^{-1}) as compared to irrigated condition (16.00, 4.35 g boll^{-1} and 56.03 g plant^{-1}, respectively).

In present study seed cotton yield of *hirsutum* cotton genotypes under different water regimes differed significantly among the genotypes and their interactions (Table 3). The mean seed cotton yield was significantly higher under rainfed condition (1283 kg ha^{-1}) as compared to irrigated condition (1261 kg ha^{-1}). The hirsutum cotton genotypes, like LRA-5166 (1491), BS-37 (1462), BS-39(1435), ARBH-1352 (1413), GBHV-182 (1404) recorded significantly high mean seed cotton yield under rain fed condition. Whereas, RAH-100 (1055), GSHV-169 (820), AKH-09-5 (1080) and GJHV-516 (1353) recorded significantly low seed cotton yield. Among the two consecutive cotton growing seasons, a decreased lint yield was observed in the first growing season due to reduced net photosynthesis under water-deficit conditions. However, no change was observed in the yield of water stress condition due to high rainfall in the next growing season as also being reported by Chastain *et al.* (2014).

The *hirsutum* cotton genotypes *viz.,* GSHV-169, BS-37, LRA-5166, AKH-09-5, TCH-1777 and BS-39 recorded less percentage reduction in rainfed over control and in contrary to this GBHV-182, PH-1060 and ARBH-1352 have shown maximum per cent reduction, hence these genotypes were considered as susceptible to water stress.

Conclusion

Based on the criteria of least per cent reduction in maximum number of parameters, five cotton genotypes *viz.,* GSHV-169, BS-37, BS-39, AKH-09-5, ARBH-1352 were identified as tolerant to water stress and these can be used to cultivate in rainfed regions. The identified drought tolerant genotypes are the good source of drought tolerant traits (SLW, water potential, biochemical parameters *etc.,),* Hence these are the potential genotypes for breeding programme to develop drought tolerant and high yielding cotton varieties.

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