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Examining the physiological relevance of cotton genotypes under drought condition

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Abstract
The study was taken to understand the physiological and biochemical mechanisms in cotton plants under drought stress. The experiment consists of four cotton cultivars, Sahana, ARBH1651, NDLH1938 and RB611 grown in the field under rainfed (stress) and irrigated conditions (non-stress). All morphological, physiological and biochemical parameters were recorded. Among the cultivars, ARBH 1651 and RB 611 showed better response to stress in terms of Relative water content, Proline content, Boll weight, seed cotton yield, Drought susceptibility index etc. Although, the cultivar Sahana recorded highest cotton yield, it showed susceptibility to stress. Hence this study concludes that the cotton cultivars ARBH 1651 and RB 611 gives better yield during rainfed condition (stress) than under irrigated conditions which indicates that these cultivars have better tolerance mechanisms to combat drought stress to some extent.

Keywords: cotton, drought stress, glycophyte, drought tolerant, drought susceptible, proline

Introduction

Agricultural production is majorly affected by various climatic conditions such as temperature, salinity and precipitation. These factors mainly control crop growth, production, productivity and various cropping systems over time (Kang *et al*., 2010; Liang *et al.*, 2016) [5, 7]. These extreme climatic conditions lead to various types of abiotic stresses such as drought, heat, cold, salinity etc. Amongst these stresses, drought stress is one of the important stresses across the globe since water is limited everywhere which causes a wide range of morpho-physiological and biochemical changes that adversely affect the development as well as the productivity of the cotton.

India occupies the largest area under cotton in the world, representing 30 to 35 percent of the total global area. It ranks second in terms of production next to China. The area under cotton in India is 122.00 lakh hectares with production of 575.00 lakh bales and productivity is 451 kg lint ha^{-1} during 2018-19 (Anon., 2019). Cotton ranks as one of the important commercial crops in India contributing nearly 70 per cent of the fibre consumption by the Indian textile industry.

Cotton production is restricted by various abiotic stresses, particularly heat and drought stress, which contributes to the substantial loss of yield world-wide (Pettigrew, 2004) [12]. Moreover, 57% of global cotton is grown in the regions with limited water condition (World Resources Institute; http://www.wri.org/). Although cotton is a glycophyte and generally grown under arid and semi arid regions, but under extreme drought condition which severely affects cotton growth and development by reducing plant height, leaf dry weight, stem dry weight, leaf area index, node number, fibre quality, canopy and root development (Loka *et al.*, 2011) [8]. Specifically, net photosynthetic rate, transpiration rate, stomatal conductance, carboxylation efficiency and water potential of cotton leaves gets decreased significantly during drought conditions (Kumar *et al.*, 2001) [6]. Recently, Hejnak *et al*. (2015) [4] studied the detrimental effects of drought stress on cotton. According to their results, 50% dry matter accumulation of *Gossypium barbadense* was limited under drought stress. Therefore in order to understand the behaviour of cotton genotype under stress and unstressed condition and to know its implications on physiological and biochemical traits, a field experiment was taken up as discussed in materials and methods.

Materials and Methods

Treatment, experimental design, and management

The present study was conducted at Agricultural Research Station, Dharwad during *kharif* 2018. The meteorological data are shown in Table 1. Four cotton cultivars were selected, Sahana, ARBH 1651, NDLH 1938 and RB611.The cultivars were sown during the month of June under rainfed (stress) and irrigated condition (non-stress). Field experiment was set up with Randomized Complete Block design with three replications and spacing in between rows and plant to plant being 60 cm and 30 cm, respectively. Other recommended agronomic practices were followed. Frequent irrigation was provided for the cultivars grown under irrigated (non-stress) condition while for the cultivars grown under stressed condition, limited water was given only when the rainfall was scanty just only to make the plants survive. Soil sample was collected at 10, 45, 90, 120 and 150 days after sowing to estimate soil moisture content by using oven dry method to ensure that the soil moisture gets reduced to 50% field capacity at the time of flowering to induce stress to the cultivars (Table 2). Recommended dosage of 40:25:25 Kg ha^{-1} NPK fertilizers were applied as per the package of practices of University of Agricultural Sciences, Dharwad. Data were recorded on randomly selected five plants in each replication for number of sympodia, number of bolls per plant, boll weight and seed cotton yield.

Table 1: The meteorological data at Agricultural Research Station, Dharwad

Months	Temperature		RH %	Rainfall (mm)	No. of Rainy days
	Maximum °C	Minimum °C			
June	28.8	21.0	81	115.4	9
July	25.8	20.6	88	128.8	19
August	25.9	20.2	87	72.2	7
September	29.6	18.9	76	69.6	4
October	31.6	18.3	66	76.8	6
November	30.5	16.3	55	34.4	2
December	28.8	14.6	61	38.4	1
Total				892.2	64

Table 2: Soil moisture status (%) of the experimental plot at Agricultural Research Station, Dharwad.

Days after sowing	% moisture in soil at 15 – 30 cm depth	
	Stress	Non-stress
10 DAS	25.00	25.64
45 DAS	21.76	24.49
90 DAS	24.12	27.92
120 DAS	10.12	27.92
150 DAS	10.64	11.87

Physiological parameters

The plant leaf samples collected from the cotton genotypes grown under stressed and non-stressed conditions were used to estimate the physiological parameters as shown in the index.

Relative water content (RWC): Leaf discs were made from five different plants collected in the plot and fresh weight was measured. Then the leaf discs were floated on deionized water in a petridish for 5 h to attain full turgidity, and then the turgid weight was determined.

Dry weight of water-soaked discs was recorded after oven drying the samples to a constant weight. The RWC was calculated using the formula:

RWC % = 100 X [(fresh weight - dry weight) ÷ (turgid weight - dry weight)]

The percent reduction in RWC over control or non-stressed plants were calculated as

(Control RWC- stress RWC)/Control RWC * 100

Biophysical parameters

The top leaves were selected to measure the net photosynthetic rate by using IRGA (Infrared gas analyzer) of LI-6400XT Portable Photosynthesis System at 50% flowering stage.

Drought susceptibility index (DSI)

Drought susceptibility index (DSI) was calculated using formula of Fischer and Maurer (1978) [3].

$$DSI = \frac{(1-Y_d/Y_p)}{D}$$

Where,

Y_d = Seed yield of the genotypes under moisture stress condition

Y_p = Seed yield of the genotypes under irrigated condition

D = Mean yield of all genotypes under moisture stress condition ÷ Mean yield of all genotypes under irrigated condition

Biochemical parameters

Leaf samples were collected from drought stressed and non-stressed plants to analyse total carbohydrate, total protein and proline content.

Total carbohydrate estimation: Total carbohydrate content was done by anthrone method (Weinmann., 1946) [13].

Total protein estimation: The leaf protein estimation was done by Lowry's method using bovine serum albumin as standard (Lowry *et al.*, 1951) [9].

Proline estimation: The colorimetric assay (Abraham *et al.*, 2010) [1] was followed for proline estimation. About 0.5 g of plant material was extracted and homogenized with 3-5 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through Whatman No.2 filter paper and volume was made upto 10 ml. Later, 2 ml of filtrate was taken in a test tube and to that 2 ml of glacial acetic acid and 2ml acid- ninhydrin was added. The test tube was heated in boiling water bath for 1 hour. Then the reaction was terminated by placing the tubes in an ice bath. After attaining room temperature, the content was transferred to a separating funnel and 4 ml of toluene was added to the reaction mixture and stirred well for 22-30 sec. The upper coloured layer was taken and the lower toluene layer was discarded and the red colour intensity was measured at 520 nm.

Results and Discussion

Number of Sympodia, Number of Bolls per plant and Boll weight

The four cotton cultivars Sahana, ARBH 1651, NDLH 1938 and RB 611 were grown under stressed and non-stressed conditions and all the morphological parameters were recorded at 90-120 DAS when the soil field capacity was less than 50% to ensure that there was a water stress during that period as given in the Table 2. The results showed that all the four cultivars had more number of sympodia and number of bolls per plant under non-stressed condition as compared to stressed condition. Since frequent irrigations were given to the cultivars grown under non-stressed condition, there was more vegetative growth which caused increased number of sympodia and also the number of bolls per plant. While the boll weight of all the cultivars was reduced under non-stressed condition, more boll weight was recorded during stressed condition. Among the cultivars, ARBH 1651 had highest boll weight of 4.92 g, while RB 611 had lowest boll weight of 4.42 g. The percent increase over non-stressed is more in ARBH 1651 followed by RB 611 which indicates that the increase in boll weight might be due to more translocation of photosynthates to the fewer bolls seen under stress in all the genotypes (Table 3).

Table 3: Number of sympodia, number of bolls per plant and boll weight of cotton cultivars grown under stressed and non-stressed condition.

	Number of sympodia			Bolls/plant			Boll weight (g)		
	Stressed	**Non-stressed**	**Mean**	**Stressed**	**Non-stressed**	**Mean**	**Stressed**	**Non-stressed**	**Mean**
Sahana	17.40	21.13	19.27	15.47	16.60	16.03	4.58	4.38	4.48
ARBH 1651	15.73	21.07	18.40	14.73	14.80	14.77	4.92	4.55	4.73
NDLH 1938	16.20	20.87	18.53	15.40	18.20	16.80	4.63	4.67	4.65
RB 611	20.67	22.47	21.57	11.93	14.93	13.43	4.42	4.08	4.25
Mean	17.50	21.38		14.38	16.13		4.64	4.42	
	Genotype	**Condition**	**Interaction**	**Genotype**	**Condition**	**Interaction**	**Genotype**	**Condition**	**Interaction**
S.Em+	1.44	1.02	0.68	1.62	1.15	0.76	3.22	2.27	1.52
C.D at 5%	6.08	4.30	2.87	6.82	4.82	3.21	13.54	9.58	6.38
CV (%)	6.06			8.67			2.90		

Seed cotton yield (kg/ha)

Seed cotton yield was recorded by weighing the seed cotton of all the cultivars grown under stressed and non-stressed conditions and represented in terms of kilogram per hectare. Among the cultivars, Sahana recorded highest seed cotton yield of 2614 kg/ha under non-stressed condition and 2466 Kg/ha under stressed condition while ARBH 1651 recorded 2072 kg/ha under stressed condition as compared to 1985 kg/ha under non-stressed condition. This indicates that although there was moderate yield reduction in ARBH 1651 cultivar as compared to Sahana, the cultivar maintained the yield even during stress. While, RB 611 recorded lowest yield under both stressed (1632 kg/ha) and non-stressed conditions (1627 kg/ha), but there was no much yield difference and it maintained the yield even during the stress. This study shows that the cultivars ARBH 1651 and RB 611 could have better buffering mechanisms to withstand stress and still be able to produce moderate yields.

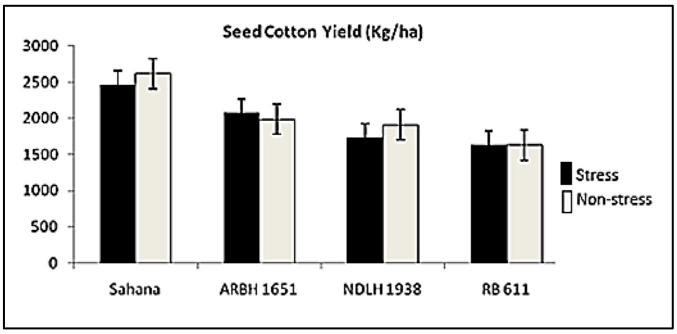

Fig 1: Seed cotton yield of cotton cultivars grown under stressed and non-stressed conditions

Drought Susceptibility Index

The relative yield performance of cotton cultivars in drought stressed and non-stressed environments can be used as an indicator to identify drought resistant varieties for drought-prone environments. Hence drought susceptibility Index was used to identify the cultivars which perform better under drought condition by maintaining good yield. Hence in this experiment it was found that Sahana had highest seed yield but it was more prone to the stress. While ARBH 1651 and RB 611 had moderate seed yield but were relatively more tolerant to the stress as compared to Sahana and NDLH 1938. This study shows that the cultivars ARBH 1651 and RB 611 were more suitable under drought conditions.

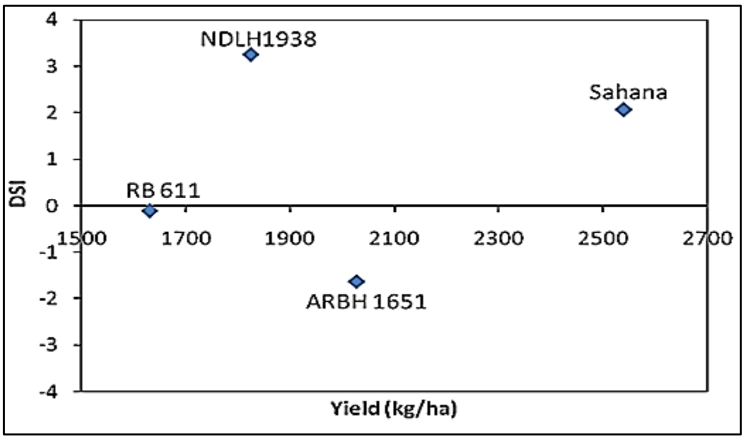

Fig 2: Biplot analysis of the Drought susceptibility index of cotton cultivars.

Relative water content (RWC)

Cotton cultivars grown in the field were subjected to stress by withholding irrigation till the 50% field capacity is reached for a period of 10 days. Relative water content of all the cultivars were recorded and compared with the non-stressed cultivars. It was found that all the four cultivars recorded highest RWC under non-stressed condition as compared to stressed condition. However under stressed condition, only ARBH

1651 showed relatively higher relative water content and the reduction in RWC over non-stressed was 6% less than the other cultivars which showed more than 8-11% reduction indicating that ARBH 1651 maintained higher water status inside the cell to function which imparted tolerance to stress. Although Sahana showed higher RWC under non-stressed condition, it drastically reduced during stressed condition indicating that Sahana could be a better cultivar only under non-stressed conditions.

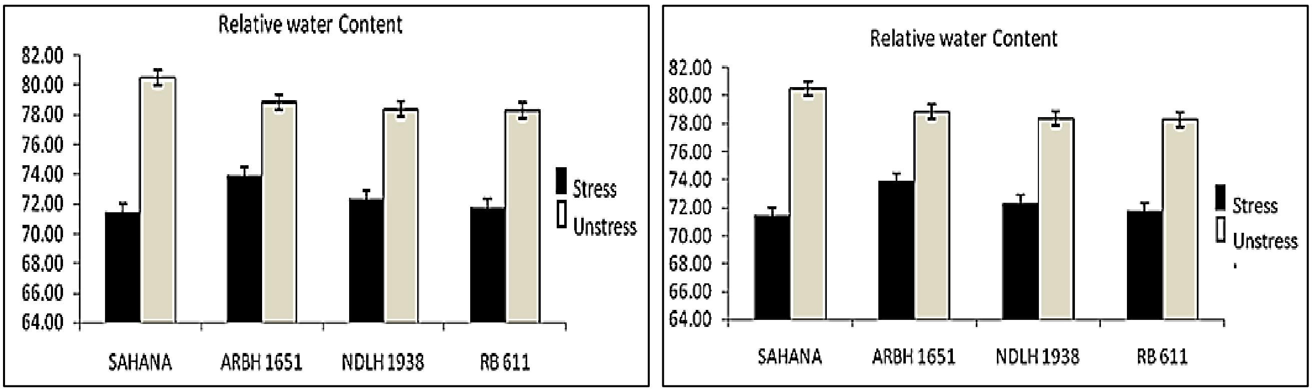

Fig 3: Relative water content and % reduction of RWC over non-stressed cotton cultivars.

Photosynthesis

The photosynthetic rate was measured in all the cultivars grown under stressed and non-stressed condition using Infra Red Gas Analyser (IRGA) and it was found that all four cultivars had higher photosynthesis under stressed condition than under non-stressed condition indicating that the cultivars retained the intact chlorophyll content which could able to take up photosynthetic activity even during the stress.

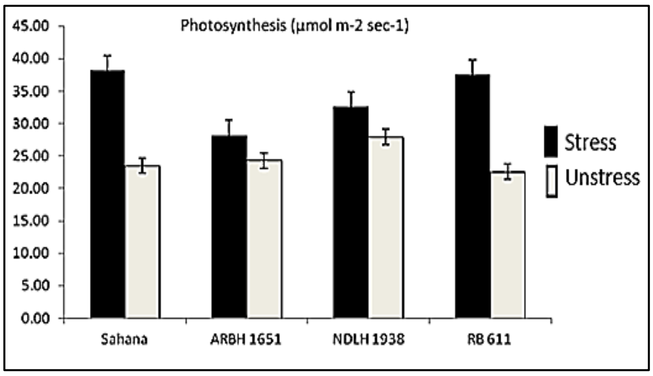

Fig 4: Photosynthesis of cotton cultivars grown under stressed and non-stressed condition.

Total Carbohydrate, Protein and Proline content

It was found that all the four cultivars recorded significantly higher carbohydrate content under non-stressed condition as compared to stressed condition which indicates that more number of photosynthates was produced during non-stressed condition in all the cultivars. Perhaps during the stress, although the cultivars showed higher photosynthetic rate but the carbohydrates produced during photosynthesis might have translocated to the sink parts or might have converted to different osmolytes needed to maintain osmoregulation during stress. Similarly the protein content of all the cultivars was higher during stressed condition than under non-stressed condition. Among the cultivars, ARBH 1651 recorded highest protein content of about 38 mg/g tissue under stressed condition as against 20 mg/g under non-stressed condition indicating that more number of stress responsive proteins or enzymes was synthesized during the stress.

When exposed to drought conditions, plants accumulate an array of metabolites, particularly amino acids such as Proline which plays a highly beneficial role in plants exposed to various stress conditions. It is one of the several small molecules classified as an osmolyte or an osmoprotectant. There is always a positive correlation between the proline accumulation and stress as illustrated in Figure 5.

Highest proline content was significantly more in Sahana, ARBH 1651 and RB 611 under stressed condition as compared to non-stressed condition indicating that the cultivars maintained high osmoregulation which is one of the drought tolerance mechanisms in plants.

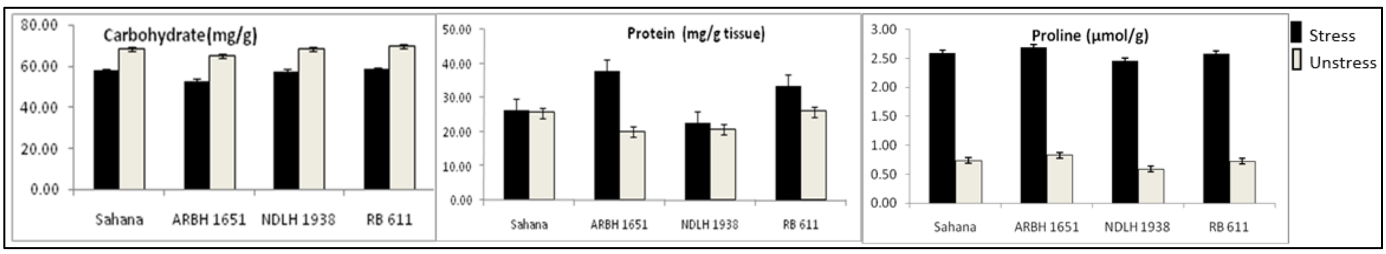

Fig 5: Carbohydrate, Protein and Proline estimation of cotton cultivars grown under stressed and non-stressed condition.

Conclusion

The present study was aimed to examine drought tolerant cotton cultivars which have an ability to perform reasonably well under drought-stressed environments which is paramount for stability in production. The cultivars ARBH 1651 and RB 611 exhibited less reduction in yield and also exhibited less Drought Susceptibility Index values, higher photosynthetic rates and Proline content under drought stress which would contribute to their suitability to drought conditions. Therefore, it can be concluded that the cotton cultivars ARBH 1651 and RB 611 could perform better under drought conditions by maintaining the physiological and biochemical activities to give higher yield. The relative yield performance of cultivars under drought stressed and non-stressed environments could be used as an indicator to identify drought-resistant varieties for drought-prone environments. Hence in future these cultivars could be used as genetic resources in breeding for improved seed cotton yield productivity specifically under drought conditions.

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Conflicts of Interest
The authors have no relevant financial or non-financial interests to disclose.
The authors have no conflicts of interest to declare that are relevant to the content of this article.
All authors certify that we have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
The authors have no financial or proprietary interests in any material discussed in this article.
Dr. Nethra. P, Dr. S.M.D. Akbar and Dr. Rajesh S. Patil declare that we do not have any conflict of Interest:

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