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Influence of Biochar, Mulch and PPFM on Stress Physiological Parameters of Cotton under Moisture Stressed Condition

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Abstract

The field experiments were conducted at Agricultural College and Research Institute, Madurai during summer and winter irrigated cotton seasons of 2016 and 2016-17. Experiments were laid out in split plot design with three replications. Moisture regimes were assigned to the main plots viz., Irrigation at IW/CPE ratio 0.4 and 0.8. The subplot comprises with moisture stress management practices viz., Cotton stalk biochar @ 5 t ha^{-1}, Cotton stalk biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1}, Cotton stalk biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1} and PPFM @ 500 ml ha^{-1} on 75 and 90 DAS, *Prosopis* biochar @ 5 t ha^{-1}, *Prosopis* biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1}, *Prosopis* biochar @ 5 t ha^{-1}+crop residue mulch @ 5 t ha^{-1} and PPFM @ 500 ml ha^{-1} on 75 and 90 DAS and Control. The stress physiological parameters observed viz., Relative leaf water content, Chlorophyll stability index and Leaf accumulated proline were greatly influenced during moisture stress period. The Maintenance of higher relative water content helps in sustaining the photosynthetic capacity of plants which ultimately contributes to higher yield. In this study higher relative leaf water content, chlorophyll stability index, lower leaf accumulated proline and higher yield of cotton was recorded in irrigation at IW/CPE to 0.8 and *Prosopis* biochar @ 5 t ha^{-1}+crop residue mulch @ 5 t ha^{-1} and PPFM @ 500 ml ha^{-1}.

Keywords: Biochar, relative water content, chlorophyll stability index, proline

1. Introduction

Cotton also known as "White Gold" is an important fibre crop in India. It plays an important role in Indian economy as the country's textile industry is predominantly based on cotton. It is cultivated in an area of 0.124 mha during 2017–2018 with production of 0.37 m bales and productivity of 542 kg ha^{-1} which is below the world average yield of 788 kg ha^{-1} (Anonymous, 2018). In cotton production, moisture stress is the primary cause for yield reduction (Singh et al., 2016). Mostly cotton cultivated in areas of hot and dry climate with limited water supply, which further down the productivity of cotton. Hence, there is a need to develop techniques to overcome the moisture deficit environments. In that way to tackle moisture stress biochar, crop residue mulch and Pink Pigmented Facultative Methylobacteria (PPFM) were well suited to

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extrude the moisture stress.

Biochar is the porous carbonaceous solid produced by thermo chemical conversion of organic materials in an oxygen depleted atmosphere which has physiochemical properties suitable for safe and long-term storage of carbon in the environment and, potentially soil improvement (Rakshit et al., 2012). Biochar application helps to improve soil moisture retention and conserve water, securing the crops against drought by its porous nature and huge surface area, which increase the porosity and had positive effect on soil water characteristics and soil fertility (Yadhav et al., 2015). Also, Molden et al. (2010) observed that in limited water conditions, influence of crop residues as mulch on crop water productivity assumed greater importance through their effects on reducing evaporation. Pink Pigmented Facultative Methylotrophs belonging to the genus *Methylobacterium* are widely explored for its plant growth promotion and induction of defence mechanisms in plant (Madhaiyan et al., 2006).

Moisture stress in cotton adversely affect both vegetative growth and major metabolic processes like photosynthesis, stomatal conductance, relative water content, chlorophyll stability index and proline content which ultimately results in reduction of biomass production and yield of cotton (Fahad et al., 2017). Reduction in relative water content results in loss of turgidity, which leads to stomatal closure and reduced photosynthetic rates. A sharp decline in relative water content under moisture stress in cotton genotypes was reported by Levi et al., 2009. Proline content of leaves increased with decline in irrigation water inferred that production of proline is probably a common response of crops under water stressed condition. Accumulation of proline in leaf is wide spread plant response of environmental stresses, including water stress. Proline acts as an osmo-protectant as well as a compatible solute (Ueda et al., 2008) and its accumulation is more under water stress condition (Reddy et al., 2004). Kar et al. (2005) studied the response of different cotton cultivars to moisture stress and found that stress apparently increased the proline accumulation. The stability of chlorophyll under moisture stress was expressed as chlorophyll stability index. Moisture stress damage the cell membrane and affect the stability of chlorophyll (Blackman et al., 1995). The research results of Vendruscolo et al. (2007) revealed that proline confers drought tolerance to wheat plants by increasing the antioxidant system rather than osmotic adjustment. Hence, to find the suitable moisture stress management practice and to study the effect of various sensitive physiological parameters this experiment was formulated.

2. Materials and Methods

The field experiments were conducted at Central Farm, Department of Farm Management, Agricultural College and Research Institute, Madurai, Tamil Nadu, India during Summer season 2016 (Feb–July) and Winter season 2016-17 (Sep- Feb) under irrigated condition. The experimental site falls under the Southern agro-climatic sub-zone of Tamil Nadu and located at 9°54′ N latitude and 78°80′ E longitude at an altitude of 147 m above MSL. The mean annual rainfall is 786.6 mm in 40 rainy days. The soil of the experimental plot was sandy clay loam in texture. low in available nitrogen (247 kg ha^{-1}), medium in available phosphorus (17.4 kg ha^{-1}) and high potassium (227 kg ha^{-1}),Organic carbon was medium (0.48%) and pH (1:2 soil water suspension) 7.4 neutral. The experiments were laid out in split plot design, replicated thrice with test variety SVPR 4. Moisture regimes were assigned to the main plots viz., Irrigation at IW/CPE ratio 0.4 (I_1) and 0.8 (I_2). The subplot comprises with moisture stress management practices *viz*., B_1-Cotton stalk biochar @ 5 t ha^{-1}, B_2-Cotton stalk biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1}, B_3-Cotton stalk biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1} +PPFM @ 500 ml ha^{-1} on 75 and 90 DAS, B_4- *Prosopis* biochar @ 5 t ha^{-1}, B_5- *Prosopis* biochar @ 5 t ha^{-1}+Crop residue mulch @ 5 t ha^{-1}, B_6 - *Prosopis* biochar @ 5 t ha^{-1}+ Crop residue mulch @ 5 t ha^{-1}+PPFM @ 500 ml ha^{-1} on 75 and 90 DAS and B_7- Control. The biochar used in these experiments were produced in traditional heap method of preparation. The *Prosopis* biochar had a bulk density and particle density of 0.44 g cm^{-3} and 0.65 g cm^{-3} respectively, with a pore space of about 32 % with high water holding capacity (111%). Cotton Stalk biochar with a bulk density and particle density of 0.39 g cm^{-3} and 0.61 g cm^{-3} respectively, had a water holding capacity of 104%.

The observations were recorded in the five fully expanded leaves of the treatment plots which is of the canopy fully exposed to sunlight were selected at random and tagged for assessing the physiological parameters. Observations were made before irrigation cycles during 70, 90 and 105 DAS of cotton crop.

Relative leaf water content (RLWC) was estimated from the method suggested by Barrs and Weatherly (1962) and result was expressed in percentage.

RLWC (%) =(Leaf fresh weight–Leaf dry weight/Leaf turgid weight–Leaf dry weight)×100

CSI was assessed according to the method suggested by Murty andMajumder (1962) and result was expressed in percentage. The leaf proline accumulation was estimated by Bates et al. (1973). The quantity of proline inthe test sample was calculated with reference to standard curve and expressed interms of µmol g^{-1} FW. The seed cotton yield was obtained from net plot area was shade dried, weighed at each picking and yields of all picking were added and calculated as kgper plot and then expressed in kg ha^{-1}. The data obtained weresubjected to statistical analysis and were tested at five per cent level of significance to interpret the treatment differences as suggested by Gomez and Gomez (2010).

3. Results and Discussion

3.1. *Relative leaf water content*

Moisture regimes had profound influence on RLWC and registered higher values during flowering (70 DAS) and comparatively less during the later stages of 90 and 105 DAS in both the seasons of experiment (Table 1). The earlier 70 DAS of cotton RLWC was higher under Irrigation at IW/CPE ratio to 0.8 (I_2) (80.4% at summer and 81.8% at winter) and lower was recorded in irrigation at IW/CPE ratio to 0.4 (I_1) with 71.3% in summer and 73.4% in winter. The same trend was followed for the later stages of 90 and 105 DAS during both the seasons. Irrigation at IW/CPE ratio to 0.8 (I_2) recorded higher values of 79.5 and 80.6% at 90 DAS and 74.6 and 76.0% at 105 DAS during summer and winter seasons respectively. Both the seasons lower values were recorded under Irrigation at IW/CPE ratio to 0.4 (I_1) at 90 and 105 DAS. The observed significant decrease in RLWC under moisture stressed condition (12 and 10% decrease at 105 DAS during summer and winter) was due to reduced absorption of water from the soil and inability to control water loss through the stomata. Similar results were reported by Kumar et al. (2012); Ananthi et al. (2013).

Table 1: Effect of Relative leaf water content to different irrigation regimes and moisture stress management practices

Treat-ments	Summer 2016						Winter 2016-17					
				90 DAS		105 DAS		70 DAS		90 DAS		105 DAS
Irrigation regimes												
I_1		71.3		70.7		65.6		73.4		72.1		68.0
I_2		80.4		79.5		74.6		81.8		80.6		76.0
Moisture stress management practices												
B_1		71.9		71.1		66.9		74.6		72.5		68.8
B_2		77.6		76.5		71.1		78.5		77.7		73.0
B_3		77.4		78.0		72.4		78.5		79.7		74.2
B_4		75.6		73.5		68.8		77.5		74.6		70.3
B_5		79.2		78.7		73.3		80.5		79.7		75.4
B_6		79.3		80.1		74.7		80.5		81.0		77.3
B_7		70.2		67.8		63.5		72.9		69.1		65.4
	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)
I	0.57	2.47	1.23	5.29	1.08	4.63	1.20	5.18	1.27	5.45	1.24	5.34
B	1.23	2.54	0.99	2.05	1.00	2.06	1.78	3.68	1.02	2.11	1.14	2.36
I×B	1.71	NS	1.79	5.58	1.69	5.02	2.62	NS	1.84	5.75	1.95	5.78
B×I	1.73	NS	1.40	2.89	1.41	2.91	2.52	NS	1.44	2.98	1.61	3.33

Among the moisture stress management practices, application of *Prosopis* biochar+crop residue mulch at 5 t ha^{-1} +foliar application of PPFM at 500 ml ha^{-1} at 75 and 90 DAS (B_6) recorded higher RLWC (74.7 and 77.3% at 105 DAS) during summer and winter seasons. This was due to the addition of biochar and mulch in the experimental soil. Biochar being porous in nature, hold the soil water content to the long and increased the field capacity (Adrian et al., 2016 and Deng et al., 2016) and addition of mulch improved the soil moisture by arresting evaporation with modified microclimate (Zhang et al., 2009, Patil et al., 2011 and Faizanullah et al., 2012). Also PPFM spray released the osmoprotectants (sugars and alcohols) on the surface of the plants. This matrix helped to protect the plants from desiccation and high temperatures (Madhaiyan et al., 2006) Though the higher values of RLWC was recorded with concentrations.

3.2. *Leaf accumulated proline*

Moisture stress induces a significant decrease in metabolic factors such as decrease in chlorophyll and enhanced accumulation of proline (Din et al., 2011). Irrigation regimes had a significant influence on the leaf accumulated proline levels. When moisture stress increased the proline levels also increased. The moisture regime at IW/CPE 0.4 recorded higher levels of proline. Minimum amount of proline (5.48 and 5.67 µg g^{-1} FW during summer and winter) was noticed with the irrigation at IW/CPE 0.8 (Table 2).

In the case of moisture stress management practices, *Prosopis* biochar at 5 t ha^{-1}+crop residue mulch at 5 t ha^{-1}+foliar application of PPFM spray at 500 ml ha^{-1} at 75 and 90 DAS (B_6) recorded lower levels of proline at 90 and 105 DAS of both summer and winter seaons. Increased proline content of leaves with decline in irrigation water inferred that

Table 2: Effect of Proline (µ mol g^{-1} FW) to different irrigation regimes and moisture stress management practices

Treatments	Summer 2016			Winter 2016-17		
		90 DAS	105 DAS	70 DAS	90 DAS	105 DAS
Irrigation regimes						
I_1	12.05	13.63	11.51	10.05	9.82	8.23
I_2	7.12	6.76	5.48	5.79	7.39	5.67
Moisture stress management practices						
B_1	10.39	10.84	10.52	8.53	10.15	8.25
B_2	8.83	10.30	7.37	7.55	7.77	6.25
B_3	8.90	9.93	6.93	7.55	7.55	5.90
B_4	9.60	10.18	9.62	7.75	9.11	7.65
B_5	8.73	9.63	7.13	6.83	7.33	6.10
B_6	8.77	8.12	5.85	6.85	6.46	5.36
B_7	11.89	12.35	12.05	10.38	11.83	9.15

	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)
I	0.46	1.98	0.08	0.33	0.32	1.37	0.18	0.77	0.39	1.66	0.24	1.04
B	0.76	1.56	0.56	1.16	0.47	0.96	0.82	1.69	0.40	0.83	0.24	0.50
I×B	1.08	NS	0.74	1.55	0.69	1.75	1.09	NS	0.65	1.86	0.40	1.16
B×I	1.06	NS	0.79	1.64	0.68	1.40	1.16	NS	0.57	1.18	0.35	0.71

the production of proline is probably a common response of crops under moisture stressed condition. The accumulation of free proline in stressed plants has been found to be an adaptive mechanism for drought tolerance. In this study also moisture stressed treatments recorded higher proline levels. This is in conformity with the findings of Lobato et al. (2008), Sampathkumar et al. (2014) and Krishnaprabu et al. (2016).

3.3. *Chlorophyll stability index (CSI)*

The chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments under moisture stress condition (Ananthi et al., 2013). The higher CSI indicates the moisture availability in leaves and it leads to increase in photosynthetic rate, dry matter production and high productivity. In this study, different treatments showed significant response to moisture stress and recorded varied range of CSI values. Severe moisture stress drastically reduced the CSI compared with enhanced water supply. The higher CSI values were recorded with irrigation at IW/CPE 0.8 (I_2) and lower was recorded with irrigation at IW/CPE 0.4 (I_1) during both the seasons of experimentation (Table 3). Moisture stress reduced CSI from 57 to 51.5% (flowering stage at 90 DAS) and 61.6 to 53.3% (boll development stage at 105 DAS) during summer and 58.6 to 50.3% (flowering stage) and 59.0 to 52.0% (boll development stage) during winter (Table 3). This may be due to degradation of chlorophyll by producing proteolytic enzymes such as chlorophyllase. In addition, under moisture deficit, the cell membrane is subjected to increase in penetrability and leakage of cell solutes, which affect the stability of chlorophyll corroborating the results of Sampathkumar et al. (2014). Besides, microscopic investigations of dehydrated cells revealed damages, including cleavage in the membrane and sedimentation of cytoplasm content.

Moisture stress management practices had a significant influence on CSI. *Prosopis* biochar at 5 t ha^{-1}+crop residue mulch at 5 t ha^{-1}+foliar application of PPFM spray at 500 ml ha^{-1} on 75 and 90 DAS (B_6) maintained relatively higher values compared with other moisture stress management practices. Younis et al. (2015) and Lyu et al. (2016) reported that biochar positively maintained chlorophyll content under water stress, which is the reason for the stability under this treatment. On the other hand, Sivakumar et al. (2017) observed lower values for CSI under severe water stress condition and reported degradation of chlorophyll due to low soil water status in Brinjal. In the present study also, severe moisture stressed plants recorded lower values for CSI, which indicated that chlorophyll is sensitive to moisture stress and disintegrated at lower RLWC.

3.4. *Seed cotton yield*

Yield is contributed by different yield parameters and any change in any one parameter as influenced by extraneous factor, will alter the yield significantly. Irrigation at IW/CPE 0.8 registered higher seed cotton yield. The yield increase was 22% (summer) and 20% (winter) as compared to irrigation

Table 3: Effect of chlorophyll stability index to different irrigation regimes and moisture stress management practices

Treatments		Summer 2016					Winter 2016-17					
			90 DAS		105 DAS		70 DAS		90 DAS		105 DAS	
Irrigation regimes												
I_1	71.3		70.7		65.6		73.4		72.1		68.0	
I_2	80.4		79.5		74.6		81.8		80.6		76.0	
Moisture stress management practices												
B_1	71.9		71.1		66.9		74.6		72.5		68.8	
B_2	77.6		76.5		71.1		78.5		77.7		73.0	
B_3	77.4		78.0		72.4		78.5		79.7		74.2	
B_4	75.6		73.5		68.8		77.5		74.6		70.3	
B_5	79.2		78.7		73.3		80.5		79.7		75.4	
B_6	79.3		80.1		74.7		80.5		81.0		77.3	
B_7	70.2		67.8		63.5		72.9		69.1		65.4	
	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)
I	0.57	2.47	1.23	5.29	1.08	4.63	1.20	5.18	1.27	5.45	1.24	5.34
B	1.23	2.54	0.99	2.05	1.00	2.06	1.78	3.68	1.02	2.11	1.14	2.36
I×B	1.71	NS	1.79	5.58	1.69	5.02	2.62	NS	1.84	5.75	1.95	5.78
B×I	1.73	NS	1.40	2.89	1.41	2.91	2.52	NS	1.44	2.98	1.61	3.33

at IW/CPE 0.4 (Table 4). The increase in seed cotton yield could be attributed to greater and consistent available soil moisture due to increased level of irrigation that resulted in better crop growth and yield components (Yang et al., 2015 and Srinivasan and Aananthi, 2017). The lower seed cotton yield with irrigation at IW/CPE 0.4 may be attributed to the decrease in synthesis of metabolites and reduction in absorption and translocation of nutrients from soil to plant under deficit moisture supply.

Higher seed cotton yield was realized with complementary alliance of irrigation regimes with moisture stress management practices recorded with *Prosopis* biochar at 5 t ha^{-1}+crop residue mulch at 5 t ha^{-1}+foliar application of PPFM spray at 500 ml ha^{-1} on 75 and 90 DAS recorded higher seed cotton yield (B_6) (Table 4). The seed cotton yield increase was 59% during summer and 61 % in winter over the control (B_7). This was evidenced from the values recorded for critical physiological characters, viz. RLWC, proline accumulation in leaf and CSI. Application of biochar as a soil amendment which exerted profound influence on soil physical properties and also biochar actas a medium for adsorption and desorption of plant nutrients. This was in consonance with the findings of Castellini et al. (2015) and Kannan et al. (2016). Mulching with foliar application of PPFM enhanced the seed cotton yield. This could be attributed to better translocation of photosynthates as reflected by the increased relative water content and chlorophyll stability index values (Rajasekar et al., 2016 and Srinivasan and Aanathi, 2017).

Table 4: Effect of different irrigation regimes and moisture stress management practice on seed cotton yield (kg ha^{-1})

Treatments	Summer 2016 seed cotton yield (kg ha^{-1})		Winter 2016-17 seed cotton yield (kg ha^{-1})	
Irrigation regimes				
I_1	1325		1399	
I_2	1619		1677	
Moisture stress management practices				
B_1	1294		1373	
B_2	1411		1484	
B_3	1517		1581	
B_4	1439		1472	
B_5	1720		1787	
B_6	1836		1963	
B_7	1084		1107	
	SEd	CD (p=0.05)	SEd	CD (p=0.05)
I	32	137	47	204
B	48	100	64	132
I×B	71	178	96	251
B×I	68	141	90	186

4. Conclusion

Physiological parameters such as leaf relative water content, leaf accumulated proline and chlorophyll stability index were more sensitive parameters to moisture stress. Irrigation at IW/CPE 0.8 and *Prosopis* biochar at 5 t ha^{-1}+crop residue mulch at 5 t ha^{-1}+foliar application of PPFM spray at 500 ml ha^{-1} on 75 and 90 DAS enrolled for its better physiology with higher yield of cotton.

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