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Biochemical and physiological characterization of grey mildew (*Ramularia areola* Atk.) disease infected cotton genotypes/hybrids

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ABSTRACT : Grey mildew disease was described as distractive to diploid cotton as compared to tetraploid cotton. Therefore, six *G. arboreum* and non-arboreum entries/variety/hybrids were selected to study the free phenol, sugars content, and photosynthesis and transpiration rate in healthy and infected leaves of cotton. Among the results highest phenol content was recorded in A 4 (4.49 mg/g), RCH 2*Bt* (4.15 mg/g) and least in 136-2 (1.41mg/g) genotypes. Reducing and non-reducing sugar content was also high in these genotypes followed by lowest in 136-2 (1.77 and 1.24 mg/g) and Jayadhar (2.39 and 1.43 mg/g) variety. The photosynthesis and transpiration rate was also high in A 1 and A 4 entries followed by least in EIPSD 4 and DDHC 11 cultivars. In the case of susceptible genotypes, drastic reduction of free phenol, sugars, photosynthesis and transpiration rate was observed in diseased leaves than the healthy once. However, ectophytic grey mildew could manage even in susceptible genotypes by spraying of 0.1 per cent difenconazole, hexaconazole and carbendazim chemicals.

Key words : Cotton, phenol, photosynthesis, *Ramularia areola*, sugars, transpiration

Grey mildew of cotton caused by *Ramularia areola* Atk. have been reported to be disease of economic significance in India as well as other part of the world. Generally grey mildew disease was distractive to diploid cotton (*Gossypium arboreum* and *G. herbaceum*) than the tetraploid cotton (*G. hirsutum* and *G. barbadense*) (Chattannavar *et al.,* 2002). Diseases brought about 20 to 30 per cent reductions in gossypol and tannin content in infected leaf. Moreover, infections due to pathogens bring about changes in plant metabolism, transpiration and chlorophyll contents of the leaf. Subsequently, photosynthetic rate and total free phenol contents are decreased significantly over a period of time.

Generally, high levels of total sugars, reducing and non-reducing sugars in the plant are stated to be responsible for disease resistance. Healthy leaves susceptible cotton produces higher amounts of total sugar than those of resistant as well as immune cotton (Chakrabarthy *et al.,* 2002). The non *Bt* genotypes (Laxmi, Abhadita, DCH 32) produces lowed amount of total phenol, reducing and non reducing sugar compared to *Bt* (RCH 2 *Bt*, JKCH 1 *Bt*, JKCH 2 *Bt*) genotypes. Further as days increased, the sugar and phenol content decreased from 90 to 120 days after sowing (Hosagoudar and Chattannavar, 2009). Therefore, six *G. arboreum* grey mildew resistant (A 1, A 4, EIPSD 4) and susceptible (ARBHA 5,

DLSA 17, 136 2) cultivars followed by none *arboreum* resistance hybrids (DCH 32, RCH 2*Bt*, Bunny 2*Bt*) and (Abhadita, DDHC 11, Jayadhar) varieties were selected to study the variation in biochemical and physiological activity of cotton.

MATERIALS AND METHODS

Sampling and extraction of plant tissue : Physiological and biochemical analysis of selected entries was done at exactly 100 days after sowing (DAS). The top healthy and diseased leaves were selected and excised in a separate polythene bag (Fig. 1). Samples were carried to the laboratory in an ice box containing ice cubes to prevent any denaturation of enzymes. One gram of tissue was weighed and made into small pieces and plunged immediately in boiling alcohol (ethanol 80%). Then it was cooled and passed through double layered muslin cloth. The pieces of the tissue were ground thoroughly in a mortar and pestle with little alcohol, and it was passed through muslin cloth. The above step was repeated once again. The filtrates were pooled and filtered through Whatmann No. 41 filter paper and made upto 10 ml with alcohol. The extract was stored in a refrigerator at 4°C.

Determination of free phenolic content : Free phenolic content of selected genotypes of

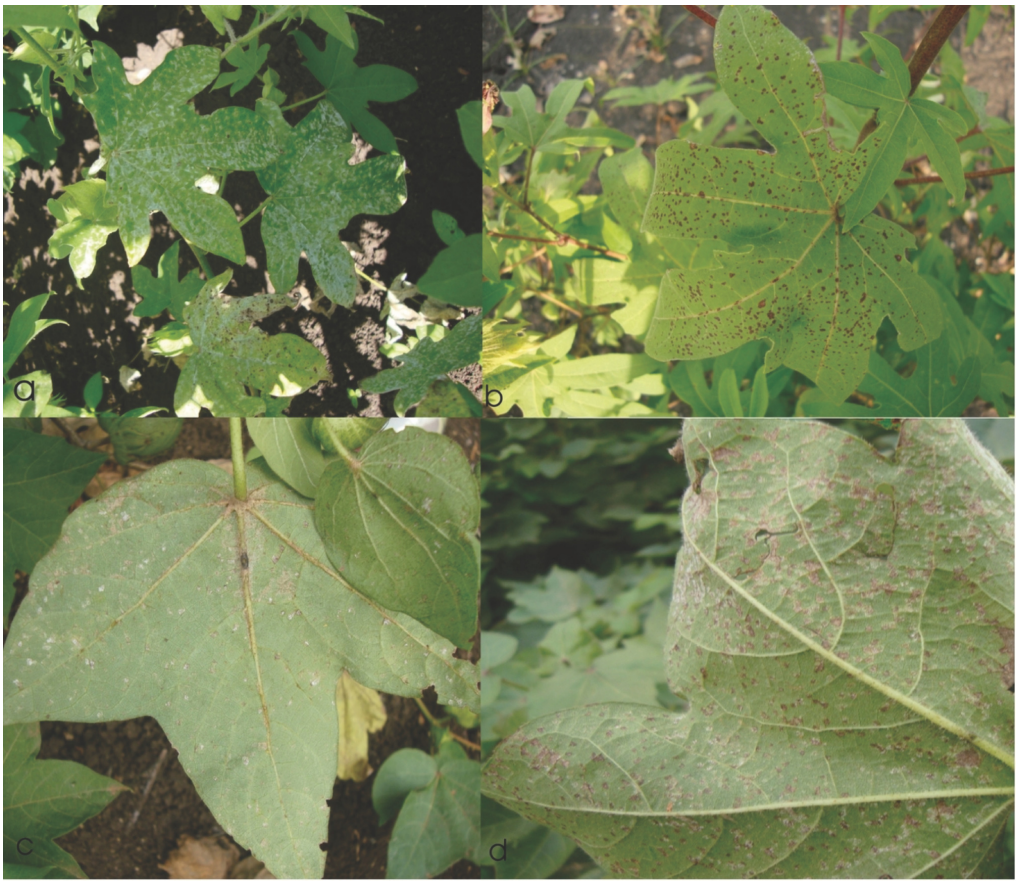

Fig. 1. Grey mildew disease infected arboreum and non-arboreum cotton genotypes/hybrids, DLSa-12 (a); EPISD-4 (b); RCH-2*Bt* (c); Bunny-*Bt* (d).

cotton was determined with Folin Ciocalteu reagent (FCR) method. A 1 ml of each alcohol extract was taken in a test tube to which 1 ml of folin-ciocalteau reagent followed by 2 ml of sodium carbonate solution (2%) were added. The tubes were shaken well and heated in a hot water bath for exactly 1 minute and then cooled under running tap water. The blue colored complex developed was diluted to 25 ml with distilled water and its absorbance was read at 650 nm in a spectrophotometer. The amount of phenols present in sample was calculated from a standard curve prepared from catechol. A standard curve was prepared to find out the concentration of phenols in the test sample and expressed as mg phenols/100g plant materials.

Estimation of reducing sugars : The reducing sugar was estimated following Nelson's modification of Somogyi's method. The 1 ml of

Fig. 2. Chemical management of grey mildew disease in DLSa 17 genotype, flowering stage (a); control (b); difenconazole sprayed (c).

each sample (alcoholic extract) was pipetted to a test tube. To each 1 ml of extract 1 ml of mixture of solution A and B was added. The test tubes were heated on a hot water bath for 20 min. The tubes were then cooled under running tap water. After cooling 1 ml of arsenomolybdate reagent was added. The above solution was diluted to 15 ml after 15 min. The absorbance of the solution was measured in spectrophotometer at 510 nm. The amount of reducing sugars was determined by using standard curve prepared with glucose.

Estimation of nonreducing sugar : Non reducing sugar was first hydrolyzed with the help of diluted hydrochloric acid. The hydrolysate was neutralized and the reducing sugar was estimated by Nelson Somogyi's method. The 1 ml of each alcohol extract was taken in a test tube and to it 1 N HCl was added. The test tubes were kept on hot water bath at 50° C for 20 min. After cooking, one drop of phenolphthalein indicator was added and mixed well. To the solution, 1 N NaOH was added drop wise till the color turned pink due to excess alkali. The excess alkali was reneutralized with 0.1 N HCl till the solution became colorless. Then the volume was made upto 5 ml. From this, 1 ml was taken and reducing sugar present in hydrolysate was estimated by Nelson Somogyi's Method. The reducing sugar in the hydrolysate was a measure of total sugar. To get the quantity of non-reducing sugar, the quantity of reducing sugar was subtracted from total sugar and it was multiplied by a conversion factor of 0.95.

Determination of photosynthesis and transpiration rate: Measurement of photosynthesis and transpiration rate was made at 100 DAS in both diseased and healthy leaves of selected genotypes by using portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA,). These measurements were made between 10 am to 12 noon.

Field experiment : A field experiment was conducted at Agricultural Research Station, Dharwad farm under rainfed conditions by using susceptible genotype DLSa 17 in order to find out suitable fungicides for controlling the disease. Total eight treatments were imposed by following randomized block design (RBD) and spray of the fungicides was given after at 60, 75,

Table 1. Effect of grey mildew disease on total phenol in different entries/hybrids of cotton

	Total free phenol (mg/g fresh weight)				
	Arboreum genotypes/entries			Non *arboreum* hybrids/varieties	
Entries	Healthy	Diseased	Entries	Healthy	Diseased
A 1	3.65	3.09	DCH 32	3.23	2.76
A 4	4.49	3.96	RCH 2*Bt*	4.15	3.69
EIPSD 4	3.83	3.14	Bunny *Bt*	3.82	3.24
ARBHA 35	3.11	1.99	Abhadita	4.31	2.71
DLSA 17	3.61	1.81	DDHC 11	2.86	1.77
136-2	2.3	1.41	Jayadhar	3.27	1.75
Genotypes (G)	1.05			0.10	
Condition (D)	0.49			0.06	
G x D	1.49			0.15	

90 days after sowing. The incidence of disease was recorded by using 0-4 scale and then these grades were converted into per cent disease indices (PDI). The cotton produce of each treatment was picked separately as and when the bolls opened and collected in cloth bags. The yield was weighed and kg/ha was computed based on the number of plants.

Statistical analysis : The experiments were conducted in replication by following the CRD design. The values were transformed to arcsin transformation. The data were subjected to the analysis of variance (ANOVA) by using the PROC GLM procedure of SAS 9.4 (SAS Institute Inc., 2016) at $p < 0.05$ was used to compare the means.

RESULTS AND DISCUSSION

Phenols have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. The total free phenolic content was significantly higher in healthy leaves than in diseased. Among the arboreum entries highest phenolic content was observed in A 4 (4.49 mg) and EIPSD-4 (3.83 mg) followed by least in 136-2 (2.30 mg) entries. However, non *arboreum* Abhadita (4.31 mg) variety showed the highest phenol followed by RCH 2*Bt* (4.15 mg) and Bunny *Bt* (3.84 mg) hybrids. As compare to healthy drastic reduction of phenol content was noticed in diseased leaves of 136 2, DLSA 17 and ARBHA 35 followed by A 1, A 4 and EIPSD 4 entries (Table 1). A resistant variety contains more phenolic than a susceptible variety. In the present study DLSA 17, ARBHA 35, 136-2, Abhadita, Jayadhar and DDHC 11 cultivar/genotypes showed the less phenol content. It's cleared that in case of susceptible cultivars the phenolic content was drastically reduced. Results of the experiment are in agreement with the findings of Chakrabarty *et al.,* (2002) and Hosagoudar and Chattannavar (2009).

Total sugars act as precursor for synthesis of phenolics and phytoalexins which play an important role in defense mechanism of plants against invading pathogens. Disease development was more whereas, the mean sugar content decreased later stage of the crop growth. The reducing and non reducing sugar content was significantly higher in non *arboreum* hybrids/varieties than the arboreum entries. The healthy leaves of A 4 (5.25 and 2.95 mg) followed by EIPSD 4 (4.33 and 2.60 mg) and A 1 (4.23 and 2.29 mg) entries showed the maximum reducing and non reducing sugars. In case of non-arboreum healthy leaves of RCH 2*Bt* (6.03 and 2.96 mg) followed by Bunny *Bt* (5.15 and 3.36 mg) and DCH 32 (5.09 and 3.44 mg) hybrids showed the highest sugar content (Table 2). Chakrabarthy *et al.,* (2002) reported that healthy leaves of the highly susceptible cotton possessed significantly higher amounts of total sugar than those of resistant as well as immune cotton. The decrease in reducing sugar and non-reducing sugar was more in infected plants. (Hosagoudar and Chattannavar, 2009)

Among the *arboreum* entries, A 1 (27.44 µmole $CO_2/m^2/S$) healthy leaves showed highest photosynthesis rate and *on par* with A 4 and 136-2 entries followed by least in EIPSD 4 (24.75 µmole $CO_2/m^2/S$). Drastic reduction in photosynthesis rate was observed in diseased leaves of ARBHA 35 (17.43 µmole $CO_2/m^2/S$) and

Table 2. Effect of grey mildew disease on sugar content in different entries/hybrids of cotton

	Arboreum genotypes/entries					Non *arboreum* hybrids/varieties			
	Reducing sugar		Non reducing sugar			Reducing sugar		Non reducing sugar	
Entries	Healthy	Diseased	Healthy	Diseased	Entries	Healthy	Diseased	Healthy	Diseased
A 1	4.23	3.83	2.29	2.07	DCH 32	5.09	4.54	3.44	3.17
A 4	5.25	4.65	2.95	2.58	RCH 2*Bt*	6.03	5.57	2.96	2.15
EIPSD 4	4.33	3.82	2.60	2.6	Bunny *Bt*	5.15	4.79	3.36	2.94
ARBHA 35	3.24	1.94	1.75	0.96	Abhadita	4.21	2.35	2.47	1.83
DLSA 17	3.55	2.50	2.49	1.26	DDHC 11	4.44	2.80	1.74	1.02
136-2	3.14	1.77	2.36	1.46	Jayadhar	3.68	2.39	2.65	1.43
Genotypes (G)	0.23		0.17			0.16		0.06	
Condition (D)	0.17		0.10			0.09		0.03	
G x D	0.42		0.25			0.23		0.09	

Sugar in mg/g fresh weight

DLSa 17(17.33 µmole $CO_2/m^2/S$) (Table 3). The varied transpiration rate was recorded in arboreum entries than the non-arboreum. Healthy leaves of A 1 (6.39 m mole of $H_2O/m^2/S$) showed highest transpiration rate followed by least transpiration rate in 136-1 (4.37 m mole of $H_2O/m^2/S$) and ARBHA 35 (4.26 m mole of $H_2O/m^2/S$) entries. Photosynthesis and transpiration rate will affect normal metabolism of the plant leading to a wide fluctuation in sugars of the plant. In the present investigation, photosynthetic rate and transpiration rate were high in healthy leaves of resistant and susceptible arboreum entries/genotype than the diseased leaves.

Total eight treatments were implemented to manage the grey mildew of cotton in *desi* cotton DLSa 17 cultivar. Difenoconazole (15.56 PDI) treatment showed less per cent disease index followed by hexaconazole (16.86 PDI) and carbendazim (17.40 PDI) (Fig. 2). However, highest disease index was

Table 3. Effect of grey mildew disease on photosynthesis and transpiration rate in different entries / hybrids of cotton

	Arboreum genotypes/entries					Non *arboreum* hybrids/varieties			
	Photosynthesis		Transpiration			Photosynthesis		Transpiration	
Entries	Healthy	Diseased	Healthy	Diseased	Entries	Healthy	Diseased	Healthy	Diseased
A 1	27.44	26.63	6.39	4.39	DCH	24.59	24.63	1.48	1.34
A 4	27.41	24.70	4.15	3.27	RCH	27.53	24.65	1.67	1.46
EIPSD 4	24.75	23.56	4.89	3.44	Bunny *Bt*	25.58	23.67	1.34	1.25
ARBHA 35	25.51	17.43	4.26	2.13	Abhadita	26.48	19.63	1.44	0.75
DLSa 17	25.50	17.33	4.87	2.54	DDHC	24.97	18.28	1.35	0.65
136-2	27.43	16.43	4.37	2.27	Jayadhar	22.43	17.31	1.35	0.73
Genotypes (G)	0.43		0.16			0.38		0.07	
Condition (D)	0.25		0.09			0.22		0.04	
G x D	0.60		0.23			0.54		0.09	

Photosynthesis rate in µ mole of $CO_2/m^2/S$ and transpiration in m mole of $H_2O/m^2/S$

Table 4. Efficacy of fungicides and bio-agents on grey mildew disease

	Treatments details	PDI	Yield (kg/ha)	B:C
T1	Hexaconazole (0.1%)	16.86 (24.25) *	1809.58	1.42
T2	Difenconazole (0.1%)	15.56 (23.24)	1880.02	1.22
T3	Tridemefon (0.1%)	30.78 (33.71)	1237.07	0.65
T4	Carbendazim (0.1%)	17.40 (24.66)	1758.11	1.37
T5	Propineb (0.2%)	28.52 (32.29)	1341.47	0.88
T6	Pyraclostrobin (5%)+Metiram (55%) WG (0.2%)	19.33 (26.09)	1730.69	0.71
T7	Iprodione (25%) +Carbendazim (25%) WP (0.2%)	23.56 (29.05)	1657.63	1.05
T8	Control	49.36 (45.18)	1033.89	0.64
	CD (p=0.05)	0.60	56.73	
	SEm±	0.20	19.09	

'*': Figures in parentheses indicate angular transformed values

recorded in tridemifon (30.78 PDI), and propineb (28.52PDI). The yield of cotton was significantly superior in all the treatments as compared to untreated control. The highest total net returns were obtained hexaconazole followed carbendazim treatments (Table 4). The three time spray of triazoles (0.1%), carbendazim (0.1%) and combi-products (0.2%) effectively controls disease even in susceptible cultivar. However Tridemefon and Propineb fungicides were not good in grey mildew disease management. The study was accordance with the findings of Chattannavar *et al.,* (2000) who reported the control of *R. areola.* Carbendazim showed maximum disease control followed by tridemorph. Chattannavar *et al.,* (2006) tested seven fungicides and reported that carbendazim manage the disease well followed by ziram and tridemorph. In the field evaluation of fungicides, propineb (0.2%) gave better control of the grey mildew disease and maximum yield of 1752.99 kg/ha (Hosagoudar, 2007).

CONCLUSION

Grey mildew of cotton was more severe in diploids than tetraploid cotton. Total free phenol, reducing and non reducing sugars, photosynthesis rate and transpiration rate were more in tetraploid cotton compare to diploid cotton genotypes/variety. However, the amount of production was almost same in healthy genotypes. However, their production fluctuated in susceptible diseased leaves of both arboreum and non-arboreum genotypes/hybrids. Spray of 0.1 per cent difenconozole, hexaconozole and carbendazim could effectively control the disease in highly susceptible variety.

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