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First report of evaluation of new world cotton genotypes (*Gossypium hirsutum*) for resistance to grey mildew disease caused by *Ramularia areola* Atk. a potential hazard for cotton belt of Odisha, India

B. Boblina[1]*, A.K. Senapati[1], S.K. Beura[2], N. Ranasingh[1], K.C. Samal[3], B.S. Nayak[4], M.K. Mishra[1], A.G. Panda[1] and D. Datta[1]

[1]Department of Plant Pathology, OUAT, Bhubaneswar, Odisha, India
[2]Department of Plant Pathology, School of Agriculture, GIET University, Gunupur, Odisha, India
[3]Department of Agricultural Biotechnology, OUAT, Bhubaneswar, Odisha, India
[4]AICRP on Cotton, RRTTS, Bhawanipatna, Odisha, India

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ABSTRACT

Cotton (*Gossypium spp.*) is a profitable commercial crop that spans over 33 million hectares in 77 countries and is a vital source of natural fibre globally. It is extensively grown in India and supports over 60 million Indians, including 6 million farmers, the majority of whom are small and marginal farmers. The current study used a randomised block design with two replications and a spacing of 90 cm x 60 cm to explore 89 cultivars from one frequently planted tetraploid species (*G. hirsutum*) in an areolate mildew hotspot in Odisha during the kharif of 2019, 2020, and 2021. The infector row technique was utilised to supplement natural disease pressure, and the severity of grey mildew was graded on a 0-4 scale. Among all the genotypes tested GSHV-159 and GISV-272 were reported to be disease free. While 32 genotypes shown only moderate resistance to the disease, 22 entries had consistently strong resistance over three years. These genotypes can be used as Grey mildew resistant donors. Twenty-four of the types tested were vulnerable to grey mildew, while the remaining nine germplasms were extremely sensitive to the fungus. These findings support cotton producers, particularly small, marginal, and tenant farmers, in selecting the best genotypes with resistance to grey mildew disease, a hazard to the environment, the economy and most importantly, sustainable crop production.

Key words: *Cotton, Grey mildew, Ramularia, AUDPC, Varietal screening*

Introduction

With 37% of the global cotton cultivation area and 24% of the global cotton production, India enjoys the status of having the most cotton - producing land. China (6423 thousand MT), India (6162 thousand MT), the United States (3181 thousand MT), Brazil (2341 thousand MT), Pakistan, Uzbekistan, Turkey, Australia, Benin, and Greece are the top cotton-producing nations globally in 2020–2021(305 thousand MT). Due to its wide variety of biological qualities, which include anti-cancer, antibacterial, anti-HIV, anti-oxidative, and male contraceptive effects, it is also used to produce gossypol in addition to fibre. In its meeting on March 22, 2022, the Committee on Cotton Production and Consumption

(COCPC) provided information on area, production, and yield in the nation for the years 2019–2020, 2020–21, and 2021–2022. For the year 2021–2022, India has 123.50 lakh hectares under cotton, with production and yield totalling to 340.62 lakh bales and 469 kg/ha, respectively. India accounts for about 12.5 million hectares of the 32.91 million ha of cotton-growing land in the world in 2020–2021. In the western and southern regions of Odisha, the high yielding long staple tetraploid cotton cultivars (*G. hirsutum*) have been introduced as an unconventional commercial crop. Currently, 1.69 lakh acres, or 2.73% of the state's total cropped area, are used for its cultivation. The current output is 4.65 lakh bales weighing 170 kg each, and the productivity is 495 kg/ha, or around 98.8% of the national output. Surveys carried out in the cotton-growing regions of Odisha revealed that a number of commonly grown cotton cultivars, including hybrids, high yielding varieties, and local varieties, were susceptible to bacterial and fungal infections. Like in almost all cotton growing states, the most frequent bacterial disease recorded happens to be Angular Leaf Blight caused by *Xanthomonas axonopodispv. malvacearum*, results in significant yield losses, followed by one of the emerging fungal diseases Grey Mildew, which in favourable weather conditions, can result in losses of up to 30% approximately in Odisha as well but had remained aloof from research point of view until the present investigation in the year 2019-2021, which happens to be the first report on the aforementioned cotton malady. The development of resistant cultivars and hybrids is a crucial and beneficial step toward integrated disease management. Development of resistant genotypes and/or hybrids requires the identification of resistant sources. In order to assess 89 cotton germplasms, including local varieties and high yielding varieties and hybrids, for resistance to the emerging threat of the crop pathogen Areolate mildew, current investigations were undertaken.

Materials and Methods

In the kharif seasons of 2019, 2020 and 2021, field experiment was carried out at the Regional Research and Technology transfer Station(RRTTS) in Bhawanipatna, Odisha (Lat. 19.924042°Long 83.149678°). In a randomised block design with two replications and a spacing of 90 cm x 60 cm, 89 cotton cultivars including 51 local varieties and 38 high yielding varieties and hybrids were sown. Utilizing the Infector Row Approach, added to the natural disease pressure (Bhattiprolu and Reddy, 2017). Each entry was positioned between rows of susceptible check MCU-5 (Shete *et al.,* 2018) (Fig. 1). Two MCU-5 border rows were also raised to surround the field. These cultivars were tested against grey mildew in unprotected environments while the crop was protected from sucking pests with prescribed pesticides. Recommended package of practices was adopted to raise the crop while no chemical control measure including herbicide, insecticide and fungicide spray was deployed and weeds were removed manually. Five randomly selected plants from each test cultivar were marked, and 10 leaves, including 3 from the bottom, 4 from the middle, and 3 from the top of each plant, were evaluated using a 0 to 4 scale (Table 1) (Sheo Raj, 1988) when grey mildew was at its highest intensity.

Based on the scores gathered, the percent disease intensity (PDI) was determined using algorithm as shown below (Wheeler, 1969):

$$\text{PDI} = \frac{\text{Sum of all the numerical ratings} \times 100}{\text{Total number of leaves scored} \times \text{Maximum disease grade}}$$

(a) 30 DAS (b) 10 DAS

Fig. 1. Line sowing of varieties forscreening against Grey Mildew disease

The test genotypes were divided into immune, highly resistant, moderately resistant, susceptible and highly susceptible groups based on Area Under Disease Progress Curve (AUDPC) value and the degree of the infection. The final disease data were considered when the infector row showed a disease score of 3 or more. AUDPC was calculated using the following formula(Madden *et al.,* 2007):

$$AUDPC=?\sum_{i=1}^{n-1}\{(Y_{i+1}+Y_i)/2\} * (t_{i+1}-t_i)$$

Y_i = Disease level at the i^{th} observation

t_i = Time in days at the i^{th} observation

$t_{i+1}-t_i$ = Time between two disease scores

n = Total number of observations/ Number of dates grey mildew was recorded

Weather condition of RRTTS, Bhawanipatna, Odisha, India

In early June of 2019, the monsoon season had already begun, with the first week alone seeing almost 72 mm of precipitation. Thus, cotton was sown beginning in the second week of June and ending in the first week of July (Table 2). A good plant stand was aided by sufficient rainfall in July and September, which was then maintained by thinning.

With 303.4 mm of rainfall in June 2020, the monsoon had already reached its peak and made it possible to sow cotton trials, which were nearly finished by the second week of July 2020. The germination was successful, and by properly filling gaps, plant populations were adequately maintained. Total rainfall for the season was 1048.0 mm, which was relatively close to the annual average rainfall of 1214.6 mm (Table 3). The cotton crop's growth was slightly hampered by the lack of late rains after the 43^{rd} SMW, but it regained following intercultural activities.

Rainfall totalled 117.6 mm in June 2021, and it occurred over the course of 4 meteorological weeks. Due to insufficient rainfall during the first week of July, the sowing began during the last week of June and with some delay it was nearly finished by the last week of the July. The plant populations were maintained by effective gap filling, and the germination was better than the previous year. A total of 829.2 mm of precipitation recorded throughout the season, which was much less than the 1214.6 mm considered to be the normal rate (Table 4). However, the crucial stages of crop growth (during September 2021) were able to receive the appropriate amount of rainfall.

Results

Among all the available disease management techniques, the use of disease-resistant cultivars effectively minimises the production cost, time, and sources of energy. Eighty-Nine test genotypes were divided into various groups in this study depending on disease response. Two cultivars, GSHV-159 and GISV-272, were recorded to be free of disease. Out of the remaining 87 genotypes 22 genotypes showed highly resistant characteristics, whereas 32 germplasms were observed to be moderately resistant to grey mildew disease (Table 5, Fig. 4.). Twenty-four of the tested varieties exhibited grey mildew susceptibility, and the remaining 9 germplasms displayed a highly susceptible response to the infection.

Discussion

For breeding programmes to use disease-resistant

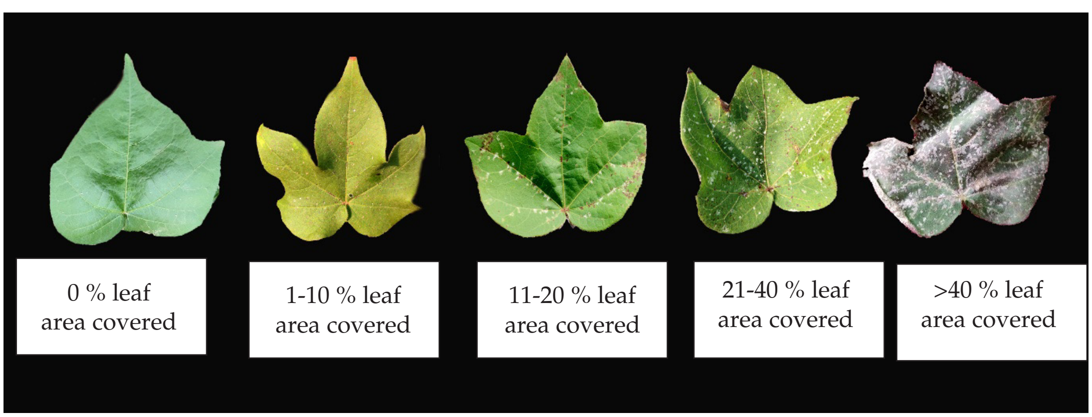

Fig. 2. Leaf area covered as per disease scale for Grey Mildew disease

Fig. 3. Profuse sporulation of *Ramularia areola* on the abaxial surface of cotton leaves

sources as donors and to encourage their cultivation in highdisease-prone locations, it is crucial to identify disease-resistant sources. Plants with long-lasting disease resistance are productive on large areas with no yield loss (Consortium, 2016). It was noted that 2% of the accessions were disease free, 25% Highly resistant, 36% of the total genotypes included in the study were found to be moderately resistant to the Grey Mildew disease, followed by 27% Susceptible to the disease, and 10% highly susceptible (Fig. 5). Bhattiprolu and Reddy (2017) studied the response of Bt cotton hybrids to foliar diseases and discovered that Ankur 2224, one of the Bt hybrids, was free of grey mildew whereas Ankur Shreyash was resistant to the disease and 69 more Bt hybrids demonstrated a mildly resistant response to grey mildew. According to Hosagoudar *et al.*

Table 1. Disease scale for Grey Mildew disease

core	Description
0	**I** = Immune, completely free from grey mildew
1	**HR**= Highly Resistant, 0-10% of leaf area covered with grey mildew disease
2	**MR**= Moderately resistant, leaf area covered up to 11- 20 % with grey mildew disease
3	**S** = Susceptible, leaf area covered up to 21- 40 % with grey mildew disease
4	**HS** = Highly Susceptible, leaf area covered > 40 % with grey mildew disease

Table 2. Monthly meteorological data of Kharif 2019

Month	Temperature(°C)		Humidity(%)		BSH(hr)	Rainfall (mm)	No. of Rainy days	Normal rainfall (mm)
	Min	Max	Max	Min				
June	27.1	37.6	71.8	55.8	20.2	110.2	6.0	240.4
July	24.8	31.7	85.1	84.0	8.4	363.5	18.0	327.7
August	24.5	30.6	88.6	84.4	8.1	408.0	15.0	355.4
September	24.4	31.4	85.7	79.9	17.6	171.6	10.0	204.6
October	22.6	30.8	86.1	73.4	23.1	69.3	6.0	74.0
November	16.9	29.4	81.8	66.2	23.8	7.0	1.0	10.9
December	14.2	27.7	81.3	63.5	18.9	0.0	0.0	1.6
				Total rainfall		1129.60		1214.6

Table 3. Monthly meteorological data of Kharif 2020

Month	Temperature(°C)		Humidity(%)		BSH(hr)	Rainfall (mm)	No. of Rainy days	Normal rainfall (mm)
	Min	Max	Max	Min				
June	25.2	34.9	73.0	66.2	20.4	303.4	11.0	240.4
July	24.5	33.3	79.2	74.8	6.8	210.8	12.0	327.7
August	24.1	30.9	84.4	81.3	15.4	218.2	17.0	355.4
September	23.7	33.2	79.2	79.0	5.4	178.8	9.0	204.6
October	21.0	31.2	83.9	75.7	18.9	133.0	11.0	74.0
November	16.1	30.2	16.1	62.1	14.8	3.8	1.0	10.9
December	11.5	29.1	69.7	51.2	26.0	0.0	0.0	1.6
				Total rainfall		1048.0		1214.6

(Hosagoudar *et al.*, 2008), JKCH-1 Bt was very prone to grey mildew, but Bt hybrids JKCH-2, JKCH-3, JKCH-4, JKCH-5, JKCH-6, Bunny and JKCH-7 were only moderately susceptible. Understanding the sources of resistance is helpful in choosing the germplasm to employ in breeding projects (Larsen and Porter, 2010). Grey mildew management depends on resistance breeding efforts, which entail locating resistance sources and introducing resistance genes into targeted genotypes.

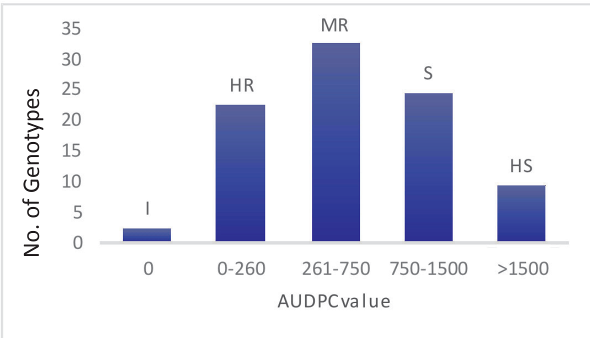

Fig. 4. Categorisation of Cotton genotypes on the basis of pooled AUDPC value in the year 2019, 2020 and 2021. AUDPC= Area under disease progress curve, I= Immune, HR- Highly resistant, MR= Moderately resistant, S=Susceptible, HS= Highly susceptible

Conflict of interest

To aid cotton breeders and geneticists in better understanding the disease, the current study is the first of its kind to be conducted in the cotton belt of Odisha, India. Fields that have become highly infested with Grey mildew as a result of the continual planting of susceptible cotton have been utilised as a natural nursery to screen and select cotton cultivars and lines for disease resistance. The cotton plant can benefit from being exposed to a variety of physical and biological settings that are likely to be found in production fields by using natural nurseries. The results derived from the present study over the span of three cropping seasons evidently prove that Infector row approach supplemented to the natural inoculum load. Higher inoculum levels are needed in controlled environments than in the field to spread the disease. Therefore, too much or too

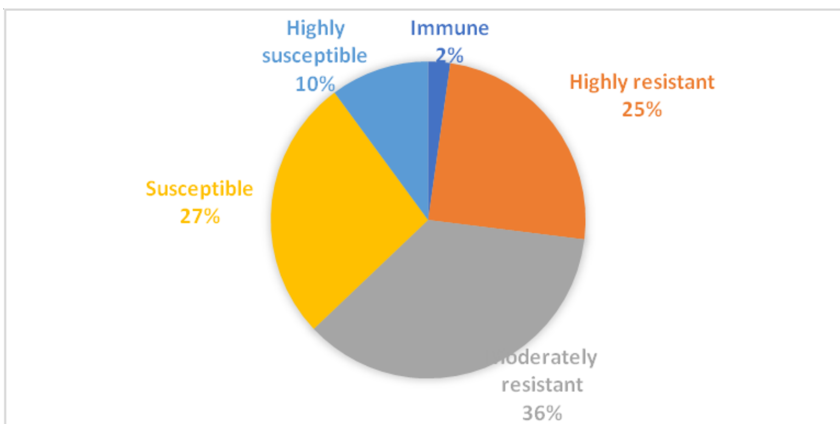

Fig. 5. Ratio of differential reactions of different cotton genotypes to Grey mildew

Table 4. Monthly meteorological data of Kharif 2021

Month	Temperature(°C)		Humidity(%)		BSH(hr)	Rainfall (mm)	No. of Rainy days	Normal rainfall (mm)
	Min	Max	Max	Min				
June	25.5	34.2	70.8	66.5	12.0	117.6	7.0	240.4
July	24.4	32.0	79.2	77.1	7.6	201.4	9.0	327.7
August	24.1	32.8	80.2	79.8	9.7	162.8	12.0	355.4
September	23.2	32.0	82.6	80.5	12.7	307.2	12.0	204.6
October	21.6	33.0	76.1	67.8	17.2	2.2	0.0	74.0
November	19.2	30.0	19.2	68.2	18.3	33.6	2.0	10.9
December	14.0	26.6	78.6	62.9	18.9	4.4	0.0	1.6
				Total rainfall		829.2		1214.6

Table 5. Screening of genotypes under field conditions in Kharif 2019, 2020 and 2021

Sl No.	Genotype	PDI (Pooled data of 2019, 2020 & 2021)	AUDPC value	Disease reaction
1.	GSHV-159	0.00	0.00	I
2.	GISV-272	0.00	0.00	I
3.	BS-2	2.16	35.92	HR
4.	BS-42	2.33	56.79	HR
5.	GSHV-177	2.41	66.29	HR
6.	BS-3-17	4.08	81.67	HR
7.	BS-6	4.33	89.63	HR
8.	BS-277	5.08	92.88	HR
9.	RAH-803	5.16	102.00	HR
10.	GJHV-516	5.33	110.13	HR
11.	BS-24	5.83	127.75	HR
12.	GJHV-500	5.83	135.00	HR
13.	BS-28	6.75	143.54	HR
14.	BS-25	7.00	150.50	HR
15.	BBC-7	7.16	171.88	HR
16.	Suraj	7.16	173.50	HR
17.	IC-359042	7.16	177.13	HR
18.	BBC-3	7.25	177.84	HR
19.	BS-30	7.41	182.13	HR
20.	BS-26	7.91	191.46	HR
21.	DHY-286	8.16	197.96	HR
22.	BBC-14	8.83	205.21	HR
23.	BS-51-1	9.41	229.13	HR
24.	TCH-1777	9.67	257.63	HR
25.	Surabhi	11.00	264.63	MR
26.	BS-40	11.08	271.67	MR
27.	GSHV-173	11.33	282.84	MR
28.	BS-41	11.41	289.21	MR
29.	BS-23	11.50	294.29	MR
30.	MCU-5-VT	11.50	295.30	MR
31.	BS-299	12.16	302.13	MR
32.	BS-1	12.25	304.67	MR
33.	GSHV-161	12.25	312.17	MR
34.	BS-35	12.33	317.46	MR
35.	BS-51	12.67	324.00	MR
36.	H-1353	12.67	356.55	MR
37.	GSHV-162	12.75	358.09	MR
38.	BS-160	12.91	369.96	MR
39.	BS-49	13.16	414.46	MR
40.	GSHV-160	13.91	425.42	MR
41.	BBC-17	14.25	441.63	MR
42.	BS-111	14.83	452.25	MR
43.	SCS-792	15.08	471.83	MR
44.	KH-140	15.33	479.46	MR
45.	H-1452	15.75	497.21	MR
46.	BS-33	16.08	504.13	MR
47.	NDLH-1939	16.25	529.63	MR
48.	RB-611	16.33	539.25	MR
49.	MCU-12	16.41	559.80	MR
50.	BS-47	17.08	581.57	MR
51.	AKH-135	17.91	602.80	MR
52.	BS-34	18.25	610.67	MR

Table 5. *Continued ...*

Sl No.	Genotype	PDI (Pooled data of 2019, 2020 & 2021)	AUDPC value	Disease reaction
53.	BBC-1	18.33	628.67	MR
54.	BS-36	18.50	669.42	MR
55.	NDLH-1938	18.75	724.67	MR
56.	BBC-9	19.41	745.29	MR
57.	GSHV-179	24.67	821.92	S
58.	SCS-1062	24.91	846.13	S
59.	BS-77-1	27.25	973.63	S
60.	NH-635	28.50	997.25	S
61.	Sumangala	28.67	1013.96	S
62.	BS-37	29.16	1055.75	S
63.	BBC-2	29.75	1065.59	S
64.	AKH-205	30.83	1078.88	S
65.	H-1316	31.33	1091.34	S
66.	BBC-8	32.33	1114.13	S
67.	BS-44	32.58	1162.42	S
68.	Anjali	32.58	1164.84	S
69.	GJHV-257	33.16	1193.21	S
70.	BBC-4	34.12	1200.09	S
71.	Supriya	34.91	1220.04	S
72.	KH-1201	35.75	1240.46	S
73.	BS-45	35.83	1286.13	S
74.	BST-1	37.41	1298.34	S
75.	BBC-6	37.50	1356.96	S
76.	BS-39	37.75	1358.05	S
77.	BS-279	38.41	1368.43	S
78.	BS-144-1	39.25	1375.85	S
79.	BGDS-1063	39.50	1399.54	S
80.	P-5430	39.83	1424.25	S
81.	BS-31	43.67	1507.79	HS
82.	BS-79	48.50	1623.44	HS
83.	BBC-13	49.00	1659.29	HS
84.	BS-277-1	52.58	1755.46	HS
85.	BS-43	53.67	1768.17	HS
86.	BS-144-2	56.67	1782.92	HS
87.	BBC-16	65.58	1975.77	HS
88.	MCU-5	66.25	2045.29	HS
89.	BS-144	66.75	2062.11	HS

little Grey mildew incidence results in limited discriminatory power when comparing genotypes for mildew resistance. The present study was carried out in the Grey mildew endemic area under field condition. Despite the known advantages of controlled environment phenotyping, it should not be viewed as a replacement for field phenotyping but rather as an additional tool that enables the acquisition of insights that are difficult to obtain under field conditions but are necessary to gain a mechanistic understanding of pathways and/or enable the training of models, provided that a well-designed experimental setup is used. Numerous factors contribute to this poor comparability, including lower light intensities and higher temperatures, particularly during the early phases of development, and frequently different plant densities in controlled environments compared to those in the field, which have an impact on total plant biomass, growth rates, leaf area, and plant design (Poorter *et al.,* 2012). Additionally, plants grown in controlled environment frequently have reduced soil volume due to pot size restrictions, which hinders root development, impacts biomass production, and alters the plants' responses to water and nutrient availability (Poorter *et al.,* 2012, Passioura, 2006). Numerous findings on source-sink

Table 6. Grouping of different Cotton genotypes according to their disease reactions

Scale	Grade	No. of genotypes	Disease reaction of varieties to Grey mildew pathogen
0	Immune	2	GSHV-159, GISV-272
1	Highly Resistant	22	BBC-14, BS-3-17, BS-30, BS-2, BS 42, BS-6, BS-24, BS-25, BS-26, BS-28, BS-51-1, BS-277, BBC-7, TCH-1777, Suraj, GSHV-177, RAH-803, IC-359042, GJHV-516, DHY-286, GJHV-500, BBC-3
2	Moderately resistant	32	BS-49, BS-41, BS-36 , BS-23 , BS-299, BS-1, BBC-9, BBC-17, BS-51, BS-47, BS-34, BS-33, BS-111, BS-160, GSHV-173, SCS-792, MCU-5-VT, BBC-1, NDLH-1938, H-1452, NDLH-1939 , AKH-135, KH-140, GSHV-161, RB-611, MCU-12, GSHV-160, GSHV-162, Surabhi, BS-35, BS-40, H-1353
3	Susceptible	24	BS-45, BBC-8, BS-44, BS-39 , BS-37, BST-1, BS-77-1, BS-144-1, BS-279, BBC-6, KH-1201, BBC-4, P-5430, BBC-2, BGDS-1063, GSHV-179, AKH-205, H-1316, NH-635, GJHV-257, Anjali, Supriya, SCS-1062, Sumangala
4	Highly Susceptible	9	BS-144, BBC-16, BS-144-2, BS-277-1, BS-43, BS-79, BS-31, BBC-13, MCU-5

interactions made under controlled circumstances in labs, climatic chambers, or greenhouses turn out to be worthless in actual agricultural practise (Fernie *et al.*, 2020). The 2 immune varieties found in this present investigation can be recommended to the breeders as a parental sources in development of resistant varieties against grey mildew. The local genotypes found to be highly resistant to the disease can be taken up for yield assessment and further recommended to the cotton growers.

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