

Original Research Article

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## Reduction in Seed Cotton Yield Corresponding with Symptom Severity Grades of Cotton Leaf Curl Disease (CLCuD) in Upland Cotton (*Gossypium hirsutum* L.)

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### ABSTRACT

Cotton leaf curl disease is the major constraint in cotton production in the Indian subcontinent. A field study (2012-2017) was conducted to assess seed cotton yield on Bt cotton cultivars analogous to the symptom rating scales (DRS) of CLCuD. The DRS is used to derive the percent disease intensity (PDI). Presently, percent disease index (PDI) for resistance screening ranges between 0.1 to 50%, corresponding to the DRS 1 to 6, which is lower than the mathematical midpoint value ranging between 8.4 to 91.8% and breeding material with good yield potential may be ignored during screening. The average yield loss corresponding to the DRS 1, 2, 3, 4, 5, and 6 was 15, 28, 38.6, 49.1, 59.1 and 72.1%. Hence, the newly proposed disease response scale would be much more justifiable and reliable for decision-making and determining the level of resistance or susceptibility in the genotypes, because the estimated severity may be much lower than the actual severity.

#### Keywords

Cotton leaf curl virus disease, Disease resistance, Symptom severity grades, Disease reaction scale, Cotton yield

#### Article Info

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### Introduction

Cotton regarded as “white gold” is one of the major cash crops in the economy of developing countries. It has a major impact on agriculture, industries, and the economy of the Indian subcontinent. It is used as raw material for indigenous textile and oil

industries and exported to many countries as cotton lint and other products of the cotton value chain. In India, cotton was cultivated in 12.24 million ha area with the production of 36.1 million bales of 170 kg each during 2018-19 (Anonymous 2019). The north zone of India covers 12% of the cotton area and 17% production (Anonymous 2019). The

demand and production of cotton have increased over the last decade, however, a lot of productivity is being reduced due to insect-pests in general and CLCuD in particular in the northern cotton zone, which is the major hurdle in achieving the full potential of the varieties and the technologies. The viral agents associated with this disease are collectively known as cotton leaf curl virus disease (CLCuD) associated begomoviruses (CABs) in association with DNA beta and alpha satellites, which belong to the genus *Begomovirus* (family *Geminiviridae*), and are transmitted by the ubiquitous whitefly (*Bemisia tabaci*) (Mansoor *et al.*, 2008; Brown *et al.*, 2015, Sattar *et al.*, 2017; Zerbini *et al.*, 2017). CLCuD symptoms are characterized by upward or downward curling of leaves, darkening of veins, vein thickening, enations that appeared in a beard-shaped and small fine leaf-like structures on the undersurface of the leaves (Briddon *et al.*, 2000, Briddon 2003; Kumar *et al.*, 2010). Severe symptoms include stunted growth of the plants, reduced flowering, and boll formation. Production is adversely affected by CLCuD which leads to deterioration of fiber quality and yield (Farooq *et al.*, 2011; Kumar *et al.*, 2011; Singh *et al.*, 2013).

The percent disease intensity (PDI) increases slowly during June and reaches up to 80% during August and September (Idris 1990; Ali *et al.*, 1995; Monga *et al.*, 1998). Monga *et al.*, (2011) have recorded disease incidence 98%, 95.4% and 93.8 during 2001, 2000 and 2006, respectively in North India.

In Pakistan, the lint yield/ha dropped down by 29.3 and 36.5% percent, respectively, during 1992-1993 and 1993-1994 as compared to 1991-1992. Seed cotton yield reduction has also been reported in Rajasthan (32.9 to 50.3%), Punjab (10.5 to 92.2%) and Haryana (39.4 to 81.4%) states of North India (Narula *et al.*, 1999; Monga *et al.*, 2001). CLCuD

affects the yield and fiber quality of cotton crops posing a major threat to cotton cultivation in Northern India (Singh *et al.*, 1994; Nateshan *et al.*, 1996; Briddon 2003; Monga *et al.*, 2004; Singh *et al.*, 2013). Seed cotton yield loss estimation studies in Bt cotton hybrids from 2008-09 to 2010-11 based on PDI ranging from 5 to 60% and disease severity grades I- IV ranged from 0.08 to 59.5%. Seed cotton yield reduction ranged from 7.2 to 80.1% at severity grades of one to four in different hybrids (Monga *et al.*, 2011). Loss estimation studies due to CLCuD on Bt cotton hybrids in Punjab and Haryana during 2009-12 ranged between 25.2 and 46.6 % (Monga *et al.*, 2012). Seed cotton yield reduction ranging from 15.7 to 56.7% was recorded during 2012-13 and 2013-14 (Monga 2014). CLCuD was reported to cause average reduction in plant height (40.6%), boll weight (33.8%) and number of bolls plant<sup>-1</sup> (72.5-73.5%), GOT% (3.93%), seed index (17.0%) and yield plant<sup>-1</sup> (64.5%) (Ahmed 1999; Mahmood *et al.*, 1996)

The variation in severity of CLCuD prevailing in the North Zone of India has been observed in farmer fields as well as research station screening trials. However, losses as per the symptom severity grades have not been properly defined. The symptom/disease rating scales (DRS) are used to derive the percent disease intensity (PDI). Work on screening of CLCuD resistance in cotton lines/cultivars is governed by the PDI corresponding to their level of resistance or susceptibility response/reaction. In the previous studies, the range of PDI which is used to decide the varieties/lines under highly resistant to the highly susceptible category also varies from the corresponding DRS and PDI. Moreover, the yield loss is in proportionate to the PDI may or may not occur, and the lines/germplasm with good yield potential may be ignored for future breeding improvement programmes.

Considering the fact that the yield loss corresponding to CLCuD symptom severity grades/scale (DRS) which is used to arrive at PDI may vary, this study was conducted for assessment of seed cotton yield analogous to the symptom severity grades at three CLCuD hot spots fields in Haryana and Punjab from 2012 to 2017 on different Bt cotton cultivars.

## **Materials and Methods**

### **Experimental sites and period**

To conduct the study on the assessment of seed cotton yield analogous to the symptom severity grades, field experiments were conducted at two locations during six consecutive years from 2012-13 to 2017-18. The experiments were conducted at two selected sites (disease hot spots) under the All India Coordinated Research Programme on Cotton (AICRP on cotton) namely Agricultural Research Station, Punjab Agricultural University, Faridkot (Punjab) (30°40'33.3"N 74°44'53.8"E), and CCS Haryana Agricultural University, Hisar (Haryana) (29°09'16.9"N 75°41'51.7"E) as part of cooperative efforts of scientists under AICRP on cotton. These sites were selected based on the CLCuD severity recorded in the past under AICRP on the cotton programme. The Bt cotton (*Gossypium hirsutum*) hybrids were selected, among the cotton cultivars recommended by the Government of the respective states.

### **Experimental design**

The field experiments were laid out in complete randomized block design (CRBD) with three replications at all the locations identified by AICRP on cotton for resistance screening against CLCuD. In each replication, the gross field plot size 250 to 500 m<sup>2</sup> was maintained. The row to row and plant to plant spacing of 67.5 x 60 cm was maintained and

crops were cultivated as per the package of practices recommended by the respective State Agricultural Department/University. Any foliar chemical treatments were not applied for disease management except for the treatments applied for the planned experimental trial.

### **Observation and data collection**

Data on CLCuD incidence were collected for estimating the percent disease incidence and disease intensity at 60, 90 and 100 days after sowing. Observations of CLCuD on 50 cotton plants from each point/ location were recorded by observing CLCuD symptoms (Akthar *et al.*, 2010; Monga, 2014). The observations were taken at 0-6 DRS (DRS) as described in Table 1.

The average grade was calculated by using the formula: Average disease rating grade = Sum of all disease rating grades/ Total number of plants, and percent disease intensity (PDI) was calculated for each entry/plot by using the following formula: PDI= (Average disease rating grades / Maximum disease rating grade) x 100.

Similarly, data on the yield characteristics viz. the number of bolls per plant, seed cotton yield were recorded from 50 cotton plants falling under each DRS. The reduction of the number of bolls per plant and seed cotton yield was computed over the lowest grade recorded during the respective cropping season. Data on CLCuD were recorded using 0- 6 scale 2012 to 2017 at PAU, Faridkot, and from 2013-2017 at CCS HAU Hisar. The pooled data of both the location for each variety as well as for the respective grades were computed to draw the inference of the effect of CLCuD on the number of bolls, seed cotton yield under each DRS. Midpoint rule or the so-called trapezoidal integration method was used to evaluate the old disease

response scale and to decide the proposed one (Campbell and Madden, 1990).

The observations were also taken for estimation of overall yield loss in each Bt hybrid during the experimental period. Disease severity of each Bt hybrid including plants with all types of symptom gradings was recorded. The CLCuD observations, number of bolls and seed cotton yield were also recorded from plants of each Bt hybrid grown in an area of 500 square meters from 2012 to 2017 at PAU, Faridkot, and from 2013-2017 at CCS HAU, Hisar.

Data were analyzed statistically and treatment effects were compared by Least Significant Difference Test (*LSD*) at 0.05% with the help of the computer program SPSS software (IBM SPSS Statistics).

## Results and Discussion

The study was conducted for the assessment of percent reduction in the number of bolls and seed cotton yield analogous to the symptom severity grades of cotton leaf curl virus disease (CLCuD) in upland cotton (*Gossypium hirsutum*) of North India. The data were collected during 2012- 2017 at CCS HAU, Hisar (Haryana) and during 2013-2017 at PAU, Faridkot (Punjab) on the number of bolls per plant and seed cotton yield (g/plant) corresponding to the CLCuD symptom severity grades/disease rating scale (DRS) in Bt cotton hybrids (BG-I, BG-II) recommended to the respective locations every year.

### Effect of CLCuD on number of bolls per plant

The "0" DRS as described by Monga (2014) was observed only during 2012, 2016 and 2017 at PAU Faridkot and the highest number of bolls per plant under DRS "0" during these

years was recorded in RCH-134 BG-I (116.2), Bioseed 6588 BG-II (54.4) and in Bioseed 6588 BG-II (58.3), respectively (Supplementary Table 1). During 2012, 2016 and 2017 the highest boll per plant corresponding to the DRS 0, 1, 2, 3, 4, 5 and 6 was recorded in RCH-134 BG-I (116.2), RCH-134 BG-I (93.2), RCH-134 BG-I (67.62), RCH-134 BG-I (51.4), Bioseed 6588 BG-II (42.0), and Bioseed 6588 (32.1). Similarly, during the year 2013, 2014, and 2015 DRS "0" was not recorded (Table 2).

The highest number of bolls per plant corresponding to the DRS 1, 2, 3, 4, 5 and 6 was recorded in RCH-134BG II (129.8), Bioseed 6588 (104.9), Bioseed 6588 (98.8), Ankur 3028 (77.2), RCH 650 (67.9), and Ankur 3028 (35.7) (*LSD* 8.317;  $P \leq 0.05$ ), respectively (Supplementary Table 1).

The DRS "0" was observed only during 2013 in the Bt cotton hybrid RCH 134 BG-I and NCS55 BG-II at CCS HAU, Hisar, Haryana while in these two varieties DRS "1" was not observed. The DRS "0" from 2014 to 2017, DRS 1 from 2015 to 2017 and DRS 2 during 2015 were not observed (Table 2).

The highest overall number of bolls per plant corresponding to the DRS 0, 1, 2, 3, 4, 5 and 6 was recorded in RCH 134 (58.5), RCH 134 (56.5), Bioseed 6588 (56.8), Ankur Jai (44.1), Ankur Jai Bt, BG-II (32.2), Ankur Jai 3028BG-II (27.8), and RCH 134 (15.3) (*LSD* 14.799;  $P \leq 0.05$ ), respectively (Supplementary Table 2).

The pooled range of reduction in the number of bolls per plant corresponding to the DRS 1, 2, 3, 4, 5 and 6 over the lowest DRS ranged from 1.5 to 32.2, 5.1 to 41.8, 10.6 to 60.2, 25.7 to 65.1, 33.6 to 76.0, and 33.3 to 97.2% (*LSD* 1.849-1.855;  $P \leq 0.05$ ), respectively (Table 2).

**Effect of CLCuD on seed cotton yield**

DRS "0" was observed only during 2012, 2016 and 2017 at ARS, Faridkot and the highest seed cotton per plant (g) corresponding to DRS 0, 1, 2, 3, 4,5, and 6 was recorded in RCH BG-I (479.2), RCH BG-I (335.2), RCH BG-I (274.6), RCH BG-I (217.8), Bioseed 6588 (196.4), Ankur 3028 (179.5), and RCH 650 (149.4) ) (*LSD* 10.129; *P* ≤ 0.05), respectively (Supplementary Table 3). Similarly, during the year 2013, 2014, and 2015 DRS "0" was not recorded. The highest seed cotton per plant (g) in corresponding to DRS 1, 2, 3, 4, 5 and 6 was recorded in Ankur Jai Bt (356.2), Bioseed 6588 (314.7), Bioseed 6588 (296.4), Ankur 3028 (231.6), RCH 650 BG-II (203.7) and Ankur 3028 (107.1), respectively (Table 3). The highest seed cotton yield per plant (g) in corresponding to DRS 0, 1, 2, 3, 4, 5 and 6 during 2013-17 at ARS, Hisar was observed in RCH 134 (205.65), RCH134 (173.91), RCH650 (223.54), RCH 650 (156.12), Ankur Jai

(110.63), Bioseed 6588 (125.71) and RCH 134 (58.65) (*LSD* 7.502; *P* ≤ 0.05), respectively ( Supplementary Table 3).

The pooled (%) reduction in seed cotton yield per plant corresponding to DRS 1, 2, 3, 4, 5 and 6 was recorded in the range from 3.4 to 30.1, 5.1 to 46.1, 10.6 to 57.2, 27.3 to 69.3, 37.3 to 83.3 and 23.2 to 97.4% (*LSD* 1.849-1.861; *P* ≤ 0.05), respectively (Table 3).

**An overall reduction in the number of bolls and seed cotton yield**

When comparing the overall reduction in the number of balls and seed cotton yield in each Bt hybrids during the experimental period, the highest seed cotton yield and number of bolls per plant were recorded during 2012 followed by 2017. The lowest reduction was recorded during 2015 followed by 2014 (Table 2 and 3).

**Table.1** Symptom and disease rating scale proposed in the past (Akthar *et al.*, 2010; Monga 2014)

S.No.	Symptoms	Disease rating scale/ (DRS) symptom severity scale	Range of percent disease intensity	Disease response
1	Complete absence of symptoms	0	0	Immune
2	Symptoms of vein thickening on a few upper leaves	1	0.1 - 10	Highly Resistant
3	Symptoms of vein thickening, cupping and curling on few upper leaves	2	11 - 20	Resistant
4	25% plant affected with vein thickening, cupping, and curling, leaf enations	3	21- 30	Moderately Resistant
5	50% plant affected with vein thickening, cupping, and curling, leaf enations	4	31 - 40	Moderately Susceptible
6	75% plant affected 5 with vein thickening, cupping, and curling, leafy enation	5	41- 50	Susceptible
7	Plants stunted severely and complete plant affected with vein thickening, cupping and curling, and leafy enation	6	> 51	Highly Susceptible



**Table.2** Pooled reduction in the number of bolls per plant under different CLCuD grades (2012-2017)

DRS (grade)	Reduction in number of bolls per plant under different CLCuD grades (%)																							
	2012				2013				2014				2015				2016				2017			
	RCH 134	RCH 134	Ankur Jai	NCS 855	RCH 134	RCH 134	Ankur Jai	NCS 855	RCH 134	RCH 134	Ankur 3028	NCS 855	MRC 7017	RC H 650	Ankur 3028	Bios eed 6588	MRC 7017	RC H 650	Ankur 3028	Bios eed 6588	MR C70 17	RC H 650	Ankur 3028	Bios eed 6588
<b>0</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>1</b>	19.8	17.4	15.9	32.2	-	-	-	-	-	-	-	-	-	-	-	-	1.5	3.8	2.1	5.3	8.1	10.3	10.1	6.2
<b>2</b>	41.8	43.0	36	28.1	21.6	24.4	5.1	17.9	8.1	20.0	13.8	15.5	12.9	6.3	12.0	5.1	5.3	9.2	12.1	6.4	9.4	15.7	17.4	13.4
<b>3</b>	51.3	60.2	48.1	43.8	41.4	35.0	29.7	35.1	26.3	31.9	21.8	30.4	19.9	10.7	16.7	10.6	13.7	16.0	18.1	14.6	22.9	25.1	22.9	23.4
<b>4</b>	55.8	65.1	62.0	51.8	61.1	52.9	44.0	51.7	36.3	39.2	47.7	44.3	25.7	27.0	31.3	36.5	28.0	29.2	30.1	24.5	35.3	39.8	31.1	41.4
<b>5</b>	73.8	76.0	66.2	62.0	64.5	63.6	56.3	63.4	57.5	60.1	50.9	65.4	35.3	33.6	42.6	47.7	42.8	44.7	41.0	38.7	45.1	49.4	37.5	52.7
<b>6</b>	89.7	86.4	79.2	82.1	79.4	79.2	73.4	71.4	83.8	97.2	95.3	93.9	60.3	52.5	64.4	76.2	45.9	48.2	43.3	41.0	60.6	65.4	48.0	65.4
<b>Over all average grade</b>	65.6	70.0	58.9	60.7	36.0	38.1	36.8	34.7	39.5	37.4	36.6	28.8	4.2	12.5	21.4	19.4	29.0	26.0	23.8	20.1	29.0	35.5	30.7	37.8

**Table.3** Pooled reduction in seed cotton yield per plant under different CLCuD grades (2012-2017)

DRS (grade)	Reduction in Seed cotton yield under different CLCuD grades (%)																							
	2012				2013				2014				2015				2016				2017			
	RC H 134	RC H 134	Ankur Jai	NCS 855	RC H 134	RC H 134	Ankur Jai	NCS 855	RC H 134	RC H 134	Ankur 3028	NCS 855	MR C70 17	RCH 650	Ankur 3028	Bios eed 6588	MR C70 17	RC H 650	Ankur 3028	Bios eed 6588	MRC 7017	RC H 650	Ankur 3028	Bios eed 6588
<b>0</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>1</b>	30.1	21.1	16.5	25.7	-	-	-	-	-	-	-	-	-	-	-	-	4.0	5.1	3.4	4.1	7.9	7.3	9.9	11.0
<b>2</b>	42.7	33.4	42.3	46.1	23.0	8.1	17.2	31.0	9.2	6.7	5.9	24.9	12.9	6.3	12.0	5.1	9.8	10.3	20.2	20.4	9.8	14.9	18.2	15.5
<b>3</b>	54.5	44.4	57.2	56.7	42.3	42.7	28.5	45.6	21.5	13.6	16.0	38.3	19.9	10.7	16.7	10.6	25.6	20.0	27.4	24.8	26.1	24.9	25.6	26.5
<b>4</b>	61.6	55.7	63.4	69.3	61.8	57.2	42.9	60.6	28.8	37.8	31.2	47.7	27.3	29.2	31.5	34.4	40.0	33.7	38.9	34.0	41.4	45.4	41.7	44.4
<b>5</b>	78.0	63.9	83.3	74.5	63.5	62.1	58.2	70.7	46.6	53.6	39.6	70.7	37.3	37.7	43.2	50.6	51.9	47.8	55.1	38.6	50.2	51.8	47.7	52.4
<b>6</b>	93.4	84.2	86.6	88.2	79.8	74.9	78.9	89.9	72.9	97.4	94.8	95.5	61.1	62.1	66.6	76.9	53.3	48.2	57.3	51.7	71.0	69.7	28.9	72.5
<b>Over all average grade</b>	69.5	64.4	73.3	62.5	39.1	45.0	33.1	47.5	28.5	18.2	17.0	35.8	10.4	16.2	11.1	17.2	29.8	26.8	31.8	28.5	35.3	39.8	32.8	39.3

**Table.4** An overall reduction in the number of bolls and seed cotton yield per plant in corresponding to the different CLCuD grades, PDI and disease response (2012-2017)

Symptoms	DRS <sup>†</sup>	Reduction % over lowest DRS				Overall reduction (%) over the lowest DRS		Percent reduction range in years where “0” was recorded		Average reduction % over “0” DRS			
		Faridkot		Hisar						Faridkot		Hisar	
		No. bolls <sup>§</sup>	SCY <sup>‡</sup>	No. bolls	SCY	No. bolls	SCY	No. bolls	SCY	No. bolls	SCY	No. bolls	SCY
<b>0. The complete absence of symptoms</b>	0	-	-			0.0	0.0	0	0	0	0	0	0
<b>1. Symptoms of vein thickening on a few upper leaves</b>	1	11.1	12.2			11.1	12.2	1.5-32.2	3.4-30.1	13.6	15.0		
<b>2. Symptoms of vein thickening, cupping and curling on few upper leaves</b>	2	17.8	20.1	12.3	11.1	16.7	18.6	5.3-43.0	9.8-46.1	24.2	28.0	10.8	16.6
<b>3. 25% plant affected with vein thickening, cupping and curling, leaf enations</b>	3	26.7	30.4	27.3	27.4	27.9	30.0	13.7-60.2	20.0-57.2	33.9	38.0	33.8	39.3
<b>4. 50% plant affected with vein thickening, cupping and curling, leaf enations</b>	4	37.4	41.3	42.5	44.4	41.3	44.3	24.5-65.1	33.7-69.3	41.0	46.7	53.9	57.6
<b>5. 75% plant affected 5 with vein thickening, cupping and curling, leafy enation</b>	5	49.6	53.0	53.6	54.3	52.9	55.3	37.5-76.0	38.6-83.3	51.4	56.1	62.1	59.6
<b>6. Plants stunted severely and complete plant affected with vein thickening, cupping and curling and leafy enation</b>	6	69.9	73.5	67.7	71.8	64.9	68.4	41.0-89.7	48.2-93.4	64.7	70.9	74.5	79.6

†: disease rating scale, ‡: seed cotton yield, § number of bolls per plant

**Table.5** An overall reduction in the number of bolls and seed cotton yield per plant in corresponding to the different CLCuD grades, DSI and midpoint value (2012-2017)

Symptoms	DRS <sup>†</sup>	DSI <sup>¶</sup> (%) corresponding to DRS	Pooled reduction (%) over “0” DRS		Disease response <sup>@</sup>	Mathematical values*			Adopted Disease Severity Index (%)	Midpoint value for adopted DSI	Proposed Disease index (%)	Midpoint value for proposed DSI
			No. bolls <sup>§</sup>	SCY <sup>‡</sup>		LCL	UCL	Midpoint				
<b>0. Complete absence of symptoms</b>	0	0	0.0	0.0	I	0	0	0	0	0	0	
<b>1. Symptoms of vein thickening on a few upper leaves</b>	1	16.7	13.6	15.0	HR	0.1	16.5	8.4	0.1 - 10	5.05	0.1-15.0	7.6
<b>2. Symptoms of vein thickening, cupping and curling on few upper leaves</b>	2	33.3	24.2	28.0	R	16.6	33.2	25.0	11 - 20	15.5	15.1-30.0	22.6
<b>3. 25% plant affected with vein thickening, cupping and curling, leaf enations</b>	3	50.0	34.6	38.6	MR	33.3	49.9	41.7	21- 30	25.5	30.1-45.0	37.6
<b>4. 50% plant affected with vein thickening, cupping and curling, leaf enations</b>	4	66.7	43.3	49.1	MS	50.0	66.6	58.4	31 - 40	35.5	45.1-55.0	50.1
<b>5. 75% plant affected 5 with vein thickening, cupping and curling, leafy enation</b>	5	83.3	54.1	59.1	S	66.7	83.3	75.1	41- 50	45.5	55.1-65.0	60.1
<b>6. Plants stunted severely and complete plant affected with vein thickening, cupping and curling and leafy enation</b>	6	100	65.9	72.1	HS	83.4	100	91.8	51<	75.5	65<	82.6

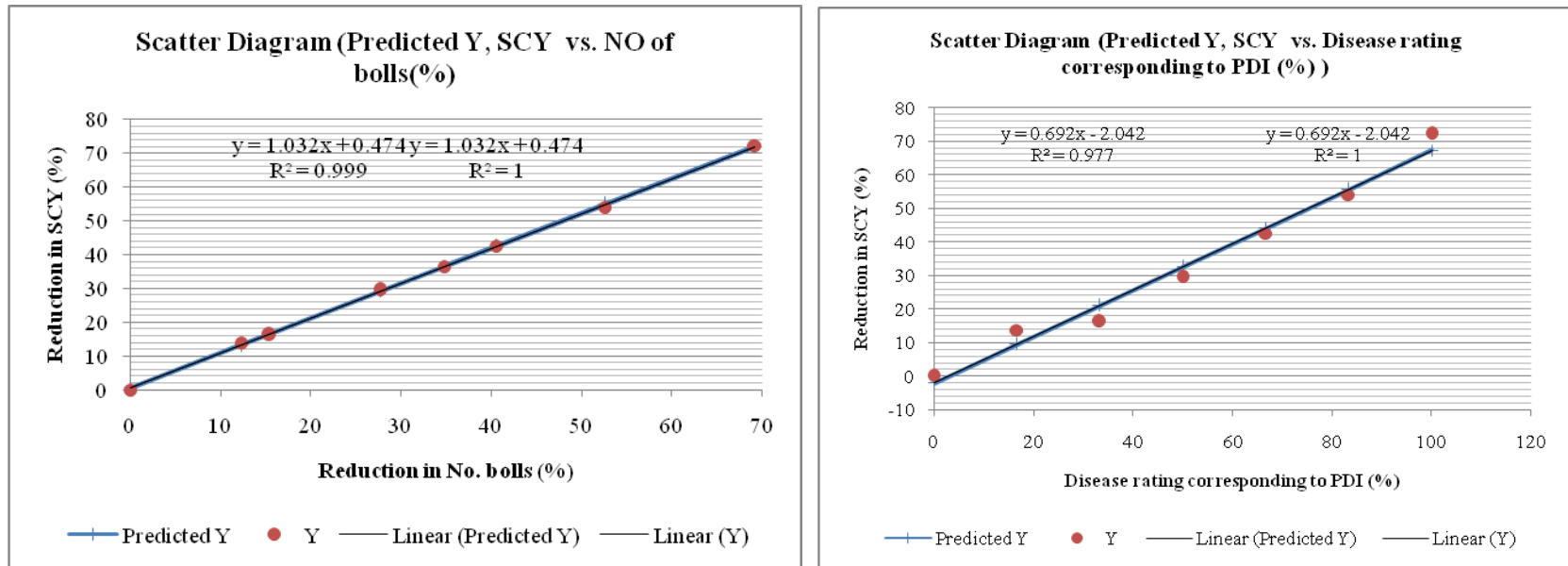
†: disease rating scale, ‡: seed cotton yield, § number of bolls per plant, ¶: disease severity index

\*Midpoint rule or so-called trapezoidal integration method (Campbell and Madden, 1990);

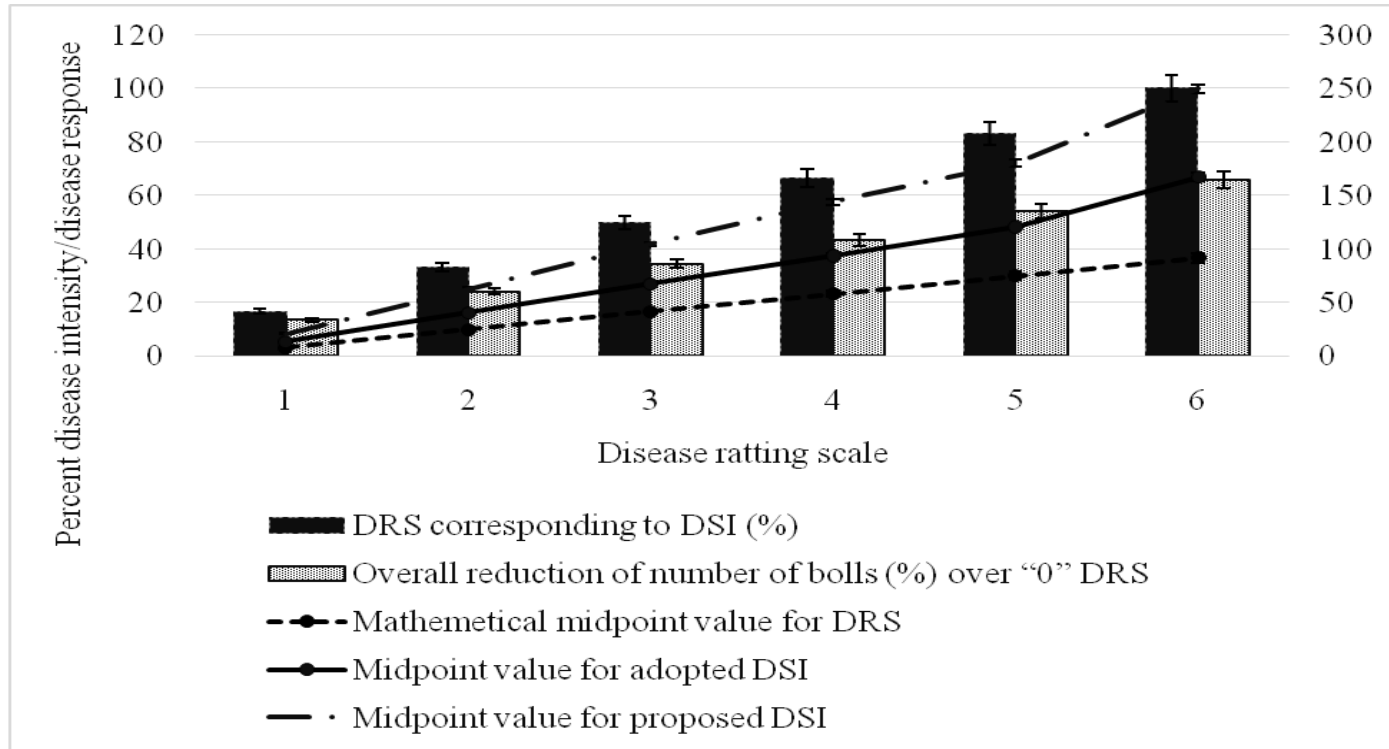
@ I=immune, HR= highly resistant, R= resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible, HS= highly susceptible



**Fig.1** Scattered diagram for regression analysis (a)  $SCY = 0.4748 + 1.0321 \times \text{No. bolls}$  (b)  $SCY = - 2.0426 + 0.6923 \times \text{PDI}$



**Figure.2** Dynamics of CLCuD rating scales on reduction in the number of bolls (NB) and seed cotton yield (SCY) (g plant<sup>-1</sup>) in different Bt cotton varieties at all the locations (pooled mean) corresponding to the PDI/DSI analogous to rating scale, mathematical midpoint value of DRS, DSI and proposed disease response during 2012-2017



The nucleotide sequence analysis of the CLCuD shared the highest level of nucleotide sequence identity (98%) with Cotton leaf curl Multan virus (CLCuMuV) during 2014 and 2015 (Qadir *et al.*, 2019) and CLCuMuV-Rajasthan strain (97% identity) during 2012 to 2014 (Biswas *et al.*, 2020). Also, as reported earlier that whitefly cryptic species Asia II-1 has mostly occurred in the cotton-growing areas of northwest India and during this study, the whitefly populations were confirmed as whitefly cryptic species Asia II 1 based on the mtCOI sequence analysis (Biswas *et al.*, 2020). These results are from other collaborative studies (Qadir *et al.*, 2019; Biswas *et al.*, 2020).

During the study period, A 0-6 disease rating scale described by Akhtar *et al.*, (2010) has been modified and used in the present studies. As in previous studies, the disease rating scale that has been generally adopted is based on percent disease intensity (PDI %). The formula used to calculate PDI has been reported by McKinney (1923). According to this formula, individual symptomatic plant rating for each genotype are summed up and divided by the number of infected plants to calculate the average grade. The corresponding PDI was calculated using the average grade divided by total rating scale i.e. 6 and multiplied by 100.

The formula to describe disease reaction/response (DSI) by Akhtar *et al* (2002, 2010) (0.1-10=HR, 10.1-20.0=R, 21-30=MR, 31-40=MS, 41-50=S, >50=HS) was found to be quite arbitrary as no scientific basis for arriving at it was presented. However, in the present studies the yield reduction vis-à-vis grades 1-6 has been presented. If all plants fall under DRS (symptom rating) "1" which is corresponding to highly resistant type symptom (DRS 1) in a particular variety/hybrid/germplasm the corresponding PDI (%) will be 16.7% (mathematical

calculation), as the disease severity index has been characterized under six-point scale, each point is thus separated by 100/6 i.e. 16.7% with yield loss about 13.6-15% (Table 4) as per our findings. According to the previously adopted disease response scale, this "1" rating or disease reaction is highly resistant which equals to 16.7% PDI falls under the previously described disease response category - 10.1-20 disease severity index 10.1-20% which is equal to the resistant response category described by of Akhtar *et al.* (2010). Similarly, this situation occurs with the other DRS corresponding to the previously adopted PDI reaction response. In the case of the "6" grade of rating scale or disease reaction HS which equals 100% PDI, it equals > 50% which is arbitrarily and without any scientific yield loss data or mathematical calculation. However, if we look at yield reduction in the case of grade six in our studies it ranges between 65.9-72.1% (Table 4).

The study conducted from 2012 to 2017, we found that the pooled reduction in the number of bolls and seed cotton yield over healthy plants (0 DRS) corresponding to DRS 1, 2, 3, 4, 5, 6 were in a similar trend. The pooled reduction in seed cotton yield over healthy plants (0 DRS) corresponding to DRS 1, 2, 3, 4, 5, 6 were 15, 28, 38.6, 49.1, 59.1 and 72.1%, respectively (Table 4). As per the mathematical calculation, the disease rating scale 1, 2, 3, 4, 5, 6 corresponding to DSI and disease response is 16.3 (HR), 33.3 (R), 50 (MR), 66.7 (MS), 83.3 (S) and 100 % (HS), respectively. Also, as per the midpoint rule or so-called trapezoidal integration method (Campbell and Madden, 1990) the midpoint value corresponding DRS 1, 2, 3, 4, 5, 6 should be 8.4, 25, 41.7, 58.4, 75.1 and 91.8, respectively. Statistically, the midpoint value for the previously adopted disease severity index looks very low then the actual i.e. 5.05, 15.5, 25.5, 35.5, 45.5, and 75, respectively.

However, the midpoint value for the proposed DSI for disease response corresponding to DRS comes to 7.6, 22.6, 37.6, 50.1, 60.1 and 82.6, respectively and is relatively nearer to the statistical midpoint value for the corresponding DSI and DRS. Also, the pooled reduction in seed cotton yield over disease-free plants (0 DRS) is almost nearer to the proposed DSI and its midpoint value corresponding DRS 1, 2, 3, 4, 5, 6 (Table 4 and 5).

The study showed that the percent reduction in seed cotton yield is more or less similar to the reduction in the number of bolls corresponding to the symptom/disease rating scales (DRS) and percent disease intensity (PDI). Similarly, the scattered diagram of the regression prediction model for seed cotton yield and the number of bolls per plant was recorded to be  $SCY=0.4748 +1.0321 \times \text{no. bolls per plant}$  ( $R^2=0.0999$ ) (Fig.1a). The scattered diagram of the regression prediction model for seed cotton yield and DRS was recorded to be  $SCY= -2.0426+0.6923 \times \text{DRS}$  ( $R^2=0.9777$ ) (Fig. 1b).

When we compare between the previously adopted PDI corresponding to different reaction grades/scales 0.1-10=HR, 10.1-20.0=R, 21-30=MR, 31-40=MS, 41-50=S, >50=HS, it is towards the lower side compared to the corresponding losses under different disease rating scale/grades. Figure 2 shows the pooled reduction in seed cotton yield and the number of bolls over the years and mid-point values for old disease response scale, mathematical PDI, and proposed disease response scale. The line graphs of three midpoint values are in alignment with the reduction in the SCY and the number of bolls per plant. The mathematical midpoint value is higher than the proposed disease response scale while the midpoint value for the old disease response scale is very lower. However, the midpoint value for the proposed disease response scale is far-far better in

terms of alignment with the yield loss is proportionate to the PDI and hence may prove to be the best to determine the resistance response of a particular genotype for future breeding improvement programmes. Thus, the previously adopted/reported scale used for resistant material screening seems to be arbitrary and is far-far lower than the new proposed scale and even the midpoint value of actual (Fig. 2, Table 5).

It is proposed that the proposed reaction/response scale would be much more justifiable and reliable for decision making for determining the level of resistance or susceptibility in the genotypes, because the estimated severity otherwise may be much higher than the actual severity. As the yield loss is in proportionate to the old resistance response scale may not occur, and the lines/germplasm with good yield potential may be ignored for future breeding improvement programmes. Hence, we propose that a midpoint value-based new resistance response scale may be utilized for decision making in future resistance screening programmes to enhance cotton production and productivity.

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