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Morpho-pathogenic variability in *Colletotrichum graminicola* isolates, causing anthracnose of sorghum (*Sorghum bicolor* (L.) Monech)

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Abstract
The present study was carried out on cultural and pathogenic variability of 8 isolates of *Colletotrichum graminicola* (Ces.) Wilson under laboratory and field condition. Isolates were recovered from sorghum anthracnose infected leaves of eight locations of four states *viz*., Rajasthan, Andhra Pradesh, Uttarakhand and Madhya Pradesh sorghum growing regions of India during 2014 and 2015. The anthracnose severity score in these locations were ranged from 4.0 to 8.0 with considerable variations in symptoms and lesions pattern. Isolates showed significant variability in cultural and morphological charters. The maximum colony diameter (88.9 mm) with highest sporulation (10.2 x 10^4 conidia/mm^2) was found in isolate Hyr Cg-08 (Hyderabad), Whereas, mean size of conidia and setae length and width was recorded from 29.7 µm to 32.4 µm x 4.8 µm to 5.3 µm and 112.8 to 134.8 x 5.4 to 5.8 µm respectively. The diameter of acervuli was ranged between195.8 to 219.4 µm. The 5 local isolates from Rajasthan were studied for their pathogenic virulence, all isolates were exhibited significant variation in latent period and anthracnose severity on 18 tested sorghum genotypes. The pooled data of two years were revealed that isolates, Udr Cg-01 (Udaipur) was the most virulent, exhibited highly susceptible reaction (7.2 to 8.5 score) on 5 sorghum lines *viz*; IS 2312, Kekri local, Rampur local, SU-45 and Maldandi and susceptible reaction on 6 sorghum lines followed by Bhl Cg-03 showed highly susceptible reaction (7.4 and 8.3) on 2 land race Rampur local and Kekri local respectively. Whereas, three lines IRAT 204, CSV 21 and PJ-1430 showed lowest disease score (1.9-2.5) across all the five isolates. Analysis of variation in anthracnose severity revealed that resistance in sorghum lines was variable and dependent on the environment, indicating potential differences in virulence of *C. graminicola* populations at different locations and over years. The resistant line can be use for further breeding programme for source of stable resistance against sorghum diseases.

Keywords: Lentil, fusarium, fungicides, evaluation, neem

Introduction
Anthracnose of sorghum (*Sorghum bicolor* (L) Monech) incited by *C. graminicola* (Ces.) Wilson (syn. *C. sublineola* Henn. In Kab. And Bubak) is one of the important sorghum melody that affect crop productivity and quality throughout the world. Pathogen *C. graminicola* was first reported on sorghum in Togo (West Africa) in 1902 (Stoop *et al*, 1982) [12]. The disease is world-wide in distribution (Pande *et al*., 1994; Ngugi *et al*., 2002) [10, 8] and has been reported from almost all sorghum growing countries of the world. Anthracnose of sorghum affects all plant parts including the stem, leaf, peduncle, inflorescence and grain (Gwary *et al*, 2002) [2] but foliar infection is more commonly observed on susceptible varieties (Thakur *et al*., 2007a) [16]. Anthracnose infects all the aerial parts of the sorghum plant and causes a total loss up to 70 per cent (Singh and Boora, 2008) [11]. There are several reports are available on the existence of Pathogenic and morphological Variability in *Colletotrichum graminicola* Isolates and in many other host combination (Thakur *et al*, 1998; Moore *et al*, 2010) [14,]. Resistance to anthracnose in sorghum is governed by single dominant genes (Tenkouano and Miller, 1993) [13] and therefore breeding for disease resistance may be effectiveness for controlling anthracnose of sorghum. Adequate knowledge of pathogen variability is required for analysis of virulence genes in the pathogen and identification of resistance genes in the host. Therefore, the studies were made on morphological, cultural and Pathogenic Variability in *C. graminicola* isolates prevelant in Rajasthan and other states of India. Iinformation on the distribution of race or pathotype in sorghum growing areas and an accurate method for identification and characterization of *C. graminicola* is necessary for effective disease management and development of host resistance in breeding programs.

Keeping these views in mind, the present experiments were designed to see cultural and pathogenic variability in *C. graminicola* isolates causing anthracnose of sorghum.

Materials and Methods

Survey, collection, isolation and symptomatology of diseased sample

To study the populations of *Colletotrichum graminicola* prevalent in different places, infected sorghum disease leaf samples were collected from the farmer's fields of Rajasthan (Udaipur, Chittorgarh, Kota, Rajsamand and Bhilwara). Other diseased samples were collected from other states of India *viz*; Sorghum Research Station, Hyderabad (NRCS) [Andhra Pradesh], Pantnagar (Uttarakhand) and Indore (Madhya Pradesh).

Data recording

Symptom of sorghum anthracnose on different places cultivars were randomly recorded on 10 plants leaves at the soft dough stage. Considerable variations in lesion size and progress pattern of spots/lesions as well as visual color of the lesions were observed. Disease severity determined by randomly scored 10 plants leaves at the soft dough stage on a standard 1-9 scale (Thakur and Mathur, 2007b) [15]. (where, 1 = no lesions, 2 = 1-5%, 3 = 6-10%, 4 - 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-50%, 8 = 51-75%, and 9 >75% leaf area covered with lesions).

Isolation and purification of the isolates

Cultures (isolates) of anthracnose pathogen (*C. graminicola*) were recovered from the samples of infected leaves collected from various eight locations. The small pieces of diseased leaves showing typical lesions of the anthracnose were cut, washed in distilled water, surface sterilized with 3 per cent sodium hypochloride for one to two minutes, and transferred on potato dextrose agar (PDA) medium in Petri-plates. The plates were incubated at 28+2°C (room temperature) for growth of culture. Subcultures were made from the periphery of the mycelial growth, which appeared after 3-4 days. The obtained culture of *C. graminicola* from different places was further purified by using single hyphal tip culturing technique and each isolates were designated (Table-1) accordingly location wise. The stock cultures of the isolates were stored on potato dextrose agar slants at 4°C and used for further studies.

Pathogenicity

Koch's postulates (Pathogenicit) of eight isolates of *C. graminicola* were proved by spray inoculating of 25-days-old plants of highly susceptible sorghum cultivar 'Kekri local under cauge-house. For this spore suspension from pure culture of different isolates was prepared separately after grown for 10-12 days in Petri-plates on PDA. Inoculations were made for each isolates seperately by spraying spore suspension ($1x10^6$ conidia ml^{-1}) with the help of a hand-held atomizer. High humidity was maintained throughout the disease development period by frequent irrigation in inoculated plants. Infection started as small flecks or light chloratic rounded spots or lesions just after 4-5 days and typical lesions/symptoms appeared 6-7 days after inoculations. Re-isolations were done from 8-10 days old lesions and the purified cultures were compared with the original ones. Identification of each isolates was confirmed by examined the detailed morphological characters of somatic and reproductive structures of *C. graminicola* with the help of standard descriptions (Holliday, 1980) [3] and were identified as *C. graminicola* (Plate-3)

Morphological and cultural variability

Five mm disc of the individual isolate of *C. graminicola* removed from the periphery of seven days-old culture was aseptically placed in the centre of the PDA plate. Five replications were maintained for each isolate in completely randomized design. The plates were incubated for growth and sporulation at 28±2°C for 10 days. The biology and variability among eight isolates of *C. graminicola* were studied for their morphological and cultural characters *viz*., colony type, colour, type of margins, colony diameter in mm were recorded after 7 days of inoculation, while rate of sporulation and, size of conidia, setae and acervuilli were measured after 10 seven days of inoculation.

Number of spore produced by each isolate was determined by removing agar-plugs (5 mm diameter) from three linear spots across the centre of the colony, which were suspended in 10 ml sterile water in glass test tubes and agitated twice for about 10 second each time on a vortex shaker to dislodge conidia. The number of conidia in the resultant suspension was determined using a haemocytometer, and expressed as number of conidia per mm^2 medium. Spore size (length and width), size of setae and acervuli measurements were taken by measuring 50 spores, setae and acervuli of each isolate using stage and ocular micrometer. Conidia, setae and acervuli development and variation in morphological characters' were studied by "damp slide culture" method.

Mass Multiplication of inoculums

The each isolates inoculums was separately multiplied on autoclaved sorghum grains in autoclavable polythene bags The sorghum grains were soaked in water overnight and washed with tap water then 150g sorghum grains were added in polythene bags and autoclaved at a pressure of 1.045 kg cm^{-2} for one hour. The overall growth culture of each isolates separately and aseptically homogenized in 10 ml sterile water with a sterilized needle and 5 ml of homogenized culture was used for seeding each polythene bag. Inoculated bags were incubated at $28+2^0$C and were shaken manually every alternate day to obtain uniform distribution of fungal growth on sorghum grains and to prevent caking. After twelve days, the grains with fungal growth and profuse sporulation were used for prepare spore suspension (inoculums) in water. The desired inoculum densities ($1x10^6$ conidia ml^{-1}) were prepared with the help of a haemocytometer.

Pathogenic variability

Pathogenic virulence variability in five local isolates of *C. graminicola viz*; Udr Cg-01, Chr Cg -02, Bhl Cg -03, Rjs Cg -04 and Kot Cg -05 recovered from Rajasthan were studied by artificially inoculated on eighteen sorghum lines (IS 8354, IS 18442, IS 2312, H-112, IRAT 204, K. Local, CSV21, Rampur Local, SSG-59-3, Pant chari 5, Raj chari 1, SU-45, Maldandi, CSV-28, PC-1080, PJ-1430, CSV-23 and IS-1077) in field condition. The test lines were grown in $3x3m^2$ plots. Each sorghum test lines were maintained in three replications for each isolates as five treatments were planned in a randomized block design. Twenty-eight-days-old plants were spray inoculated with ($1x10^6$ conidia ml^{-1}) containing tween-20 (1

ml/l) with each isolate of *C. graminicola*. The inoculation was done in the late evening, followed by a heavy irrigation to provide adequate moisture for infection. Leaf wetness upto 3 days was maintained by spraying water on plants thrice a day.

Data recording

Twenty plants were randomly selected for latent period from each replication per plot basis. Observation for latent period/incubation period (time in hours for development of first chlorotic or necrotic lesion) was started from 3rd day of inoculation up to 5days. Disease severity was scored on randomly selected 20 plants per plot from each replication after 20 days of inoculations on 1-9 disease rating scale (Thakur and Mathur, 2007b) [15]. Based on disease reaction, entries were designated as HR (highly resistant-no symptoms), score (0.0-1.0), R (resistant), score (1.1-3.0), MR (moderately resistant) score (3.1-5.0), S (susceptible) disease score (5.1-7.0) and HS (highly susceptible) disease score (7.1-9.0). To designating virulence, isolates showing disease reaction of HR, R or MR on a sorghum line were considered avirulent and those showing S or HS were considered as virulent. The mean values of these on per plot basis were used for analysis.

Results and Discussion.

Survey and Disease severity.

Eight locations of four states were surveyed and eight isolates of *C. graminicola* were recovered and designated accordingly their localities as Udr Cg-01 (Udaipur), Chr Cg-02 (Chittorgarh), Bhl Cg-03 (Bhilwara), Rjs Cg-04 (Rajasamand), Kot Cg-05 (Kota) from Rajasthan state and Cg-06 (Indore), Pan Cg-07 (Pantnagar) and Hyd Cg-08 (Hydrabad). In surveyed areas anthracnose severity was recorded in moderate to severe form with 4.0 to 8.0 disease scores on different sorghum cultivars. The highest disease severity with (8.0) mean disease score was recorded from Udaipur on cultivar kekri local, followed by (7.5 score) on CSV 17 and SSV 84 from Bhilwara and Hydrabad respectively. While least disease severity with (4.0 score) was recorded from Kota on SPV 1333 sorghum cultivar (Table: 1). Prevalence of sorghum anthracnose in various sorghum growing regions with the incidence of 40-50% on different sorghum cultivars has been reported by (Naugi *et al*. 2002 and Mathur *et al*. 2002) [8, 6].

Symptoms variability

Eight samples collected from various places were exhibited considerable variations in anthracnose symptoms and lesions pattern on their respective sorghum cultivars. Samples from Udaipur initial exhibited reddish and round spots/lesions in scattered pattern on susceptible cultivar Kekri local, later enlarged and collapse with each other. A dark red black concentric ring of acervuli was also observed in the lesions (Plate-1, fig.1). In Chittorgarh, on locai cultivar leaf samples the initial lesions were varied with coffee colour and round smaller than UDR Cg-01, acervulli were formed in the center with dark black colour (Plate-1, fig.2). Bhilwra samples showed reddish and small dotted spots on sorghum cultivar CSV-17, but acervuli were not observed. The initial lesion on local cultivars from Rajsamand were formed small rounded with dark reddish colour without acervuli in the centre (Plate-1, fig-4.) The initial lesions on SPV 1333 from Kota were exhibited small dotted with dark reddish colour and later become elongated with black concentric ring of acervuli (Plate-2, fig-5).

The anthracnose symptoms in other states leaf samples were also varied with each other from Indore leaves samples initially exhibited very small dots and reddish colour on K-108-2, later become large rounded to elongated and collapse. The fully developed lesions were bigger than those on other samples (Plate- 2, fig.6). Samples of Pantnagar **e**xhibited Coffe colored round lesions on local land race (Plate-2, fig.7), while symptoms from NRCS, Hyderabad were differed with small dotted with dark reddish colour and later become in irregular shape on SSV 84 (Plate-2 fig.8). Although, in all samples sunken necrotic dry straw tissue reflected in the center with light yellowish with black concentric ring of acervuli in the lesions. Similar studies was conducted by (Pande *et al*, 1991) [9] with nine isolates collected from variou locations of India and reported variation in leaf symptoms on different sorghum lines with elliptical to elongate lesions/spots with yellow, red halos and acervuli in the centre of the lesion. (Lingaraju and Hegde, 1977) [5] reported that initial symptoms were appeared on the leaves as small, circular or elliptical spots with red, purple or brown color margins with whitish or purple centers. As progress the disease spots enlarged slightly with characteristic black dots in the centre and found that these dots represented the acervuli of the fungus. The variation in the anthracnose symptoms described from different locations indicates the possibility of variation in the pathogen as well as differences in response of host genotypes of sorghum.

Cultural and morphological characters of eight isolates

The eight isolates of *C. graminicola* showed considerable variations in cultural and morphological characters. The maximum mean fungal growth colony diameter (88.9 mm) was recorded for the isolate Hyr Cg-08. This was followed by 86.7 mm in Rjs Cg-04 and 84.5 mm in Ind Cg-06 (Indore). The least mean colony diameter (43.4 mm) was of isolate Bhl Cg-03 Table. 1; Plate-4). The maximum number of conidia in the culture was produced by isolate Hyr Cg-08 (10.2×10^4 conidia/mm^2), followed by Udr Cg-01 (9.9×10^4) and Pan Cg-07 (9.4×10^4). The least sporulation (6.9×10^4 conidia/mm^2) was recorded in isolate Rjs Cg-04 at 40X ((Table. 1&2).

The margin/shape and colour of the culture of different isolates varied from fluffy culture with many concentric rings (zonation) which were very prominent towards the middle that varied in colour dirty whitish black (Hyr Cg-08). In Ind Cg-06 oppressed culture without concentric ring and dirty white later turn gray. In Rjs Cg-04 surface of culture showed many concentric rings (zonation) which was very prominent towards the middle and colour was dirty white. In Pan Cg-07 surface of culture have concentric ring (zonation) with fluffy margin that varied in colour from dirty white to gray (Table-1; Plate-4).

In Udr Cg-01 the growth was fluffy with cottony without concentric ring, no regular margin and dirty whitish gray in colour. In Chr Cg-02 oppressed and faint growths without concentric ring and initially showed white later became dirty whitish gray colour. In Kot Cg-05 it was fluffy cottony growth in center of culture without concentric ring and dirty white in colour. While in Bhl Cg-03 the growth was developed fluffy without concentric ring and whitish in colour (Table- 1, Plate-4).

Variation in size of conidia, setae and acervuli

All the eight isolates of *C. graminicola* showed significant variations in conidia, setae and acervuli morphology. The mean length and width of conidia in different isolates ranged from 29.7 (26.4-33.8) µ to 32.4 (28.8-36.3) µ x 4.8 (4.3-5.4) µ to 5.3 (4.7-5.9) µ. The mean length and width of setae in different isolates ranged from 112.8 (100.4-126.3) to 134.8 (120.0-151.0) x 5.4 (4.8-6.0) to 5.8 (5.2-6.5) µ and diameter of acervuli in different isolates ranged 195.8 (174.3-219.3) to 219.4 (195.3-245.7) µ. Table.3.

The maximum length and width of conidia were of the isolate Udr Cg-01, which measured 32.4 (28.8-36.3) µ x 5.3 (4.7-5.9) µ. Conidia of Bhl Cg-03 were next, measuring 32.2 (28.7-34.5) µ x 5.2 (4.6-5.8) µ in size. The smallest size of conidia isolate was of Kot Cg-05 that measured 29.7 (26.4-33.8) x 4.8 (4.-5.4) µ. In the remaining five isolates the conidial size was intermediate of these two. Size of conidia from Hyderabad isolate (Hyr Cg-08) measured 30.5 (27.1-34.2) x 4.9 (4.4-5.5) µ, while in Ind Cg-06 isolate size of conidia were 30.2 (26.9-35.2) x 4.9 (4.4-5.5) µ in size (Table-4,). The maximum length and base width of setae were of the isolate Udr Cg-01, which measured 134.8 (120.0-151.0) µ x 5.8 (5.2-6.5) µ. Setae of Pan Cg-07 was next, measuring 131.2 (116.8-146.9) µ x 5.7 (4.9-6.4) µ in size. The smallest size was of setae of isolate Kot Cg-05 that measured 112.8 (100.4-126.3) x 5.4 (4.8-6.0) µ. In the remaining five isolates the setae size was intermediate of these two. In Bhl Cg-03 it was 127.8 (113.7-143.1) x 5.6 (5.0-6.3) µ, in Chr Cg-02 the mean size of setae was 121.8 (108.4-136.4) x 5.5 (4.9-6.2) µ, setae of Ind Cg-06 were 120.7 (107.4-135.2) x 5.5 (4.9-6.2) µ, setae of Hyr Cg-08 measured 118.4 (105.4-132.6) x 5.6 (5.0-6.3) µ and setae of Rjs Cg-04 measured 117.5 (104.6-131.6) x 5.4 (4.8-6.0) µ in size at 100X (Table- 2). The causal agent of anthracnose on cereals, including maize and sorghum has been long regarded as *C. graminicola* (Holliday, 1980) [3]. However, the perfect state, *Glomerela graminicola* Politis, is known for both sorghum and maize isolates of *C. graminicola* (Vaillancourt and Hanau, 1992) [17]. It was only after rDNA sequences supported the separation from *C. graminicola* that *C. sublineolum* became generally accepted species for sorghum isolates (Singh and Boora, 2008) [11]. More concerted efforts, using more number of isolates and wide range of growth conditions are needed to generate perfect states of sorghum isolates that will help in understanding genetics of this important pathogen.

Despite these variations, the size of conidia agreed well with the standard description of conidia (26.1-38.8 × 4.9-5.2µm) of *C. gaminicola* (Thakur and Mathur, 2007). The micrometrical data of present study confirm the reports published by Kumar (2006) [4] and thus the present results are on the similar line of published literature.

Pathogenic variability among the isolates of *c. graminicola* on sorghum lines

The pooled data of two years *i.e.*, 2014 and 2015 revealed that isolates showed considerable variations and significant difference in mean latent period, and disease severity on different test lines. The latent period of different isolates on lines ranged from 75 to 120 hrs across the eighteen differentials. The shortest mean latent period across the eighteen lines was 94.8 hrs by isolate Udr Cg-01 (Udaipur), followed by 98.0 hrs in Bhl Cg-03 (Bhilwara). The mean latent period for Chr Cg-02 (Chittorgarh) was 101.2 hrs and for Kot Cg-05 (Kota) it was 104.0 hrs. The longest mean latent period 107.3 hrs was of Rjs Cg-04 (Rajsamand).

Among the lines longest mean latent period (incubation period) (116.4 hrs) across the five isolates was in CSV-21 followed by 115.2 hrs in IRAT-204, 114.6 hrs in IS 8354, 113.4 hrs in PJ-1430, 112.8 hrs in CSV-23, 112.2 hrs in SSG 59-3, 111.0 hrs in PC-1080, 104.4 hrs in CSV-28, 102.6 hrs in IS 18442 and Pant chari-5, 102.6 hrs in IS-1077, 98.4 hrs in H-112 and 93.0 hrs in Raj chari-1, 90.6 hrs was in Maldandi, 90.0 hrs in SU-45, 81.6 hrs in IS-2312, The shortest mean latent period (78.6 hrs) was on K local followed by 79.8 hrs in Rampur local (Table 3).

Similarly, pooled data of two years 2014 and 2015 revealed that the isolates Udr Cg-01 (Udaipur) was the most virulent compare to all other isolates, as it caused highly susceptible (HS) reaction (7.1 to 8.5 score) on five of the eighteen lines. The disease score was 8.5 on K local,7.9 on Rampur local, 7.7 on IS 2312, 7.3 on Maldandi and 7.1 on SU-45. This isolate exhibited susceptible reaction (5.2 to 6.8 score) on Raj chari-1 (score 6.8), H-112 (score 6.1), IS18442 (score 5.4), IS-1077 (score 5.3), Pant chari-5 and CSV-28 (score 5.2) and moderately resistant (MR) reaction on CSV-23 (score 3.8), SSG 59-3 (score 3.3), IS 8354 and PC-1080.This isolate gave resistant (R) reaction (score 2.9) on PJ-1430, score 2.5 on IRAT 204 and score 2.2 on CSV 21. Isolate showed as a virulent on K local, Rampur local, IS 2312, Maldandi, SU-45, Raj chari 1, H-112, IS 18442, IS-1077 and Pant chari 5 and show avirulent on CSV-21, IRAT 204, PJ-1430, PC-1080, IS 8354, SSG-59-3, CSV-23 and CSV-28 (Table 4).

This was followed by Bhl Cg-03 (Bhilwara) which caused highly susceptible (HS) reaction with mean diseases score 8.2 on K local and Rampur local (score 7.4). This isolate caused susceptible (S) reaction on IS 2312 (score 6.8), SU-45 and Rajchari1 (score 6.6), Maldandi (score 6.4), H-112 (score 5.4), Pant chari-5 and IS 1077 (score 5.1) and moderately resistant (MR) reaction on IS 18442 (score 4.8), CSV-28 (score 4.5) and CSV-23 (score 3.7). This isolate resistant (R) reaction (score 3) on SSG 59-3, score 2.9 on IS 8354, score 2.8 PC-1080, score 2.6 on PJ-1430, score 2.3 on IRAT 204 and score 2.0 on CSV-21. Isolate showed as a virulent on K local, Rampur local, IS 2312, SU-45, Raj chari 1, Maldandi, H-112, IS-1077 and Pant chari 5 and show avirulent on CSV-21, IRAT 204, PJ-1430, PC-1080 IS 8354, SSG-59-3, CSV-23, CSV-28, IS 18442.The isolate Chr Cg-02 (Chittorgarh) gave highly susceptible (HS) reaction with disease score 7.7 on K local. It gave susceptible (S) reaction on Rampur local (score 7.0), IS 2312, SU-45 and Raj chari-1 (score 6.5), Maldandi (score 5.8) and H-112 (score 5.3). It gave moderately resistant (MR) reaction on Pant chari-5 and IS-1077 (score 4.7), IS18442 (score 4.6), CSV-28 (score 3.4), CSV-23 (score 3.3) and it gave resistant (R) reaction (score 2.7) on SSG 59-3, score 2.5 on IS 8354, score 2.4 on PJ-1430 and PC-1080, score 2 on IRAT 204 and score 1.6 on CSV 21. Isolate showed as a virulent on K local, Rampur local, IS 2312, SU-45, Raj chari 1, Maldandi and H-112 and show avirulent on CSV-21, IRAT 204, PC-1080, PJ-1430, IS 8354, SSG-59-3, CSV-23, CSV-28, IS 18442, IS-1077 and Pant chari 5 (Table 4).

Isolate Kot Cg-05 (Kota) gave highly susceptible (HS) reaction with disease score 7.3 on K local. It gave susceptible (S) reaction on Rampur local (score 6.7), IS 2312 and Raj chari-1 (score 6.2), Maldandi (score 5.6) and SU-45 (score 5.3). It gave moderately resistant (MR) reaction on IS 1077 (score 4.8), H-112 (score 4.5), IS 18442 (score 4.4), Pant chari-5 (score 4.3), CSV-28 (score 3.2) and CSV-23 (score 3.1). It gave resistant (R) reaction (score 2.6) on PC-1080, score 2.5 on IS 8354 and SSG 59-3, score 2.3 on PJ-1430, score 2.1 on IRAT 204 and score 1.6 on CSV-21. Isolate

showed as a virulent on K local, Rampur local, IS 2312, Raj chari-1, Maldandi and SU-45 and avirulent on CSV-21, IRAT 204, PJ-1430, SSG-59-3, IS 8354, PC-1080, CSV-23, CSV-28, Pant chari-5, IS 18442, H-112 and IS 1077.

Isolate Rjs Cg-04 (Rajsamand) caused susceptible (S) reaction with disease score 6.6 on K local, Rampur local (score 6.4), IS 2312 (score 5.9), Raj chari-1 (score 6.0), Maldandi (score 5.5) and SU-45 (score 5.2). It gave moderately resistant (MR) reaction on H-112 (score 4.4), IS 18442 (score 4.1), Pant chari-5 (score 4.0) and IS 1077 (score 3.9). It gave resistant (R) reaction (score 2.7) on CSV-28 and CSV-23, score 2.4 on PC-1080, score 2.3 on SSG-59-3, score 2.0 PJ-1430, score 1.9 on IS 8354, score 1.7 on IRAT 204 and score 1.5 on CSV-21. Isolate showed as a virulent on K local, Rampur local, IS 2312, Raj chari-1, Maldandi and SU-45 and avirulent on CSV-21, IRAT 204, IS 8354, PJ-1430, SSG-59-3, PC-1080, CSV-23, CSV-28, IS 1077, Pant chari-5, IS 18442 and H-112. Among the sorghum lines, highest mean disease score 7.6 was recorded on K local across the five isolates, followed by Rampur local (score 7.1), IS 2312 (score 6.6), Raj chari-1 (score 6.4), SU-45 and Maldandi (score 6.1), H-112 (score 5.1), IS 18442, IS 1077 and Pant chari-5 (score 4.7), CSV-28 (score 3.8), CSV-23 (score 3.3), SSG-59-3 (score 2.7), IS 8354 and PC-1080 (score 2.6), PJ-1430 (score 2.4) and IRAT 204 (score 2.1) and least mean disease score 1.8 was observed on CSV-21 across the five isolates. The differences in latent period, and disease severity among the five isolates across the eighteen test lines were statistically significant (P=0.05) for the isolates, test lines and interaction in isolate x test lines (Table 4). The information's on sorghum anthracnose resistance has been reported to be governed by dominant genes (Tenkouano and Miller, 1993) [13], but there is no information on the number of resistant loci, as well as for the genes in lines showing partial/moderate resistant (MR) reaction or delayed disease development (longer latent period). Thus, based on the disease reaction, these five isolates of *C. graminicola* have been assigned in to two pathotypes (i) Pathotype Udr Cg-01 (Udaipur) and Bhl Cg-03 (Bhilwara) and (ii) Pathotype Chr-02 (Chhitorgarh), Rjs Cg-04 (Rajasamnd) and Kot Cg-05 Kota). Similar studies on pathogenic variability have also been reported in six isolates of *C. gaminicola* on 16 sorghum lines based on cultural characters and pathogenicity (Thakur *et al*., 1998) [14]. (Moore *et al*,2010) evaluated the reaction of 10 sorghum hybrids to eight *C. sublineolum* pathotypes collecting anthracnose and recorded different levels of resistance available in the current grain sorghum hybrids to different pathotypes

Table 1: Variation in anthracnose severity and Cultural charters in different Isolates (*C. graminicola*) of different locations.

Place and Sorghum cultivar	Isolates	Mean disease score	Cultural and Colony Characters			
			Diameter * (mm) 10th days	Sporulation* (x10⁴/mm² medium)	Growth characters	Colour variation
Udaipur (Rajasthan) Kekri local	Udr Cg-01	8.0	57.8	9.9	Fluffy with cottony growth with no concentric ring, no regular margin	Dirty whitish grey
Chittorgarh (Rajasthan) Local land race	Chr Cg-02	6.5	45.5	7.4	Appressed and faint growth with no concentric ring	Initial white later become dirty whitish gray
Bhilwara (Rajasthan) CSV17	Bhl Cg-03	7.5	43.4	8.5	Fluffy growth with no concentric ring	Whitish gray
Rajasmand (Rajasthan) Local land race	Rjs Cg-04	6.0	86.7	6.9	Surface of culture with many concentric rings with zonation which are very prominent towards the middle	Dirty white
Kota (Rajasthan) SPV 1333	KotCg-05	4.0	48.2	7.5	Fluffy cottony growth in center of culture with no concentric ring	Whitish gray
Indore (Madhya Pradesh) K-108-2	Ind Cg-06	5.5	84.5	8.3	Appressed growth with no concentric ring	Dirty white later become gray
Pantnagar (Uttarakhand) Local land race	Pan Cg-07	7.0	77.6	9.4	Surface of culture with many concentric rings with zonation and fluffy margin	Dirty white later become gray
Hyderabad (Andhra Pradesh)	Hyr Cg-08	7.5	88.9	10.2	Fluffy culture with many concentric rings with zonation which are very prominent towards the middle	Dirty whitish black
S.Em±			1.426	0.216		
CD (P=0.05)			4.10	0.62		

* Mean of five replications

Table 2: Variations in conidia, setae and acervuli morphology of different isolates of *C. graminicola*

Isolates	Size of Conidia (µm)*				Size of Setae (µm)*				Accervulus Diameter (µm)*	
	Length		Width		Length		Base Width			
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Udr Cg-01	32.4±1.5	28.8-36.3	5.3±0.2	4.7-5.9	134.8±6.2	120.0-151.0	5.8±0.27	5.2-6.5	219.4±10.2	195.3-245.7
Chr Cg-02	31.8±1.7	28.3-35.6	5.1±0.3	4.5-5.7	121.8±6.5	108.4-136.4	5.5±0.29	4.9-6.2	201.3±10.7	179.2-225.5
Bhl Cg-03	32.2±1.7	28.7-34.5	5.2±0.3	4.6-5.8	127.8±6.6	113.7-143.1	5.6±0.29	5.0-6.3	210.8±10.9	187.6-236.1
Rjs Cg-04	30.8±1.6	27.4-33.3	4.9±0.3	4.4-5.5	117.5±6.1	104.6-131.6	5.4±0.28	4.8-6.0	198.7±10.4	176.8-222.5
Kot Cg-05	29.7±1.4	26.4-33.8	4.8±0.2	4.3-5.4	112.8±5.2	100.4-126.3	5.4±0.25	4.8-6.0	195.8±9.1	174.3-219.3
Ind Cg-06	30.2±1.6	26.9-35.2	4.9±0.3	4.4-5.5	120.7±6.4	107.4-135.2	5.5±0.29	4.9-6.2	206.5±11.0	183.8-231.3
Pan Cg-07	31.4±1.6	27.9-34.2	5.1±0.3	4.1-6.0	131.2±6.8	116.8-146.9	5.7±0.33	4.9-6.4	214.3±12.2	182.8-242.4
Hyd Cg-08	30.5±1.6	27.1-34.2	4.9±0.3	4.4-5.5	118.4±6.2	105.4-132.6	5.6±0.29	5.0-6.3	208.4±10.9	185.5-233.4
S.Em±	0.09		0.02		0.36		0.02		0.64	
CD (P = .05)	0.25		0.05		1.01		0.05		1.79	

* Mean no. of 50 conidia, setae and accervuli and ±SD of mean value

Table 3: Latent period of five isolates of *C. graminicola on* different sorghum lines (Pooled value-2014 and 2015)

Host Line	Isolate (Latent period in hrs)*					Mean
	Udr Cg-01	ChrCg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	
IS 8354	108	114	114	120	117	114.6
IS 18442	96	102	102	108	105	102.6
IS 2312	75	81	78	90	84	81.6
H-112	90	96	96	108	102	98.4
IRAT 204	108	117	111	120	120	115.2
K local	75	78	75	84	81	78.6
CSV – 21	114	117	114	120	117	116.4
Rampur local	75	78	78	84	84	79.8
SSG 59-3	108	114	108	117	114	112.2
Pant chari 5	99	96	102	108	108	102.6
Raj chari 1	87	93	90	99	96	93.0
SU-45	81	90	87	99	93	90.0
Maldandi	81	96	84	96	96	90.6
CSV – 28	96	108	96	114	108	104.4
PC-1080	102	111	111	117	114	111.0
PJ-1430	108	114	111	120	114	113.4
CSV-23	108	114	111	117	114	112.8
IS-1077	96	102	96	111	105	102.0
Mean	94.8	101.2	98.0	107.3	104.0	
		S.Em±	CD (*P*=0.05)			
Host line		0.79	2.19			
Isolates		0.42	1.16			
Host line x isolate		1.76	4.91			

* Mean of three replication, 20 plants per replication

Table 4: Disease severity of sorghum lines to five isolates of *C. graminicola* (Pooled value-2014 and 2015)

Host Line	Disease severity (Disease score 1-9 scale) *					Mean
	Udr Cg-01	Chr Cg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	
IS 8354	3.1(MR) (A)	2.5 (R) (A)	2.9 (R) (A)	1.9 (R) (A)	2.5 (R) (A)	2.6 (R)
IS 18442	5.4 (S) (V)	4.6 (MR) (A)	4.8 (MR) (A)	4.1 (MR) (A)	4.4 (MR) (A)	4.7 (MR)
IS 2312	7.7(HS) (V)	6.5 (S) (V)	6.8 (S) (V)	5.9 (S) (V)	6.2 (S) (V)	6.6 (S)
H-112	6.1 (S) (V)	5.3 (S) (V)	5.4 (S) (V)	4.4 (MR) (A)	4.5 (MR) (A)	5.1 (S)
IRAT 204	2.5 (R) (A)	2.0 (R) (A)	2.3 (R) (A)	1.7 (R) (A)	2.1 (R) (A)	2.1 (R)
K local	8.5 (HS) (V)	7.7 (HS) (V)	8.2 (HS) (V)	6.6 (S) (V)	7.3 (HS) (V)	7.6 (HS)
CSV – 21	2.2 (R) (A)	1.6 (R) (A)	2.0 (R) (A)	1.5 (R) (A)	1.6 (R) (A)	1.8 (R)
Rampur local	7.9 (HS) (V)	7.0 (S) (V)	7.4 (HS) (V)	6.4 (S) (V)	6.7 (S) (V)	7.1 (HS)
SSG 59-3	3.3 (MR) (A)	2.7 (R) (A)	3.0 (R) (A)	2.3 (R) (A)	2.5 (R) (A)	2.7 (R)
Pant chari 5	5.2(S) (V)	4.7 (MR) (A)	5.1 (S) (V)	4.0 (MR) (A)	4.3 (MR) (A)	4.7 (MR)
Raj chari 1	6.8 (S) (V)	6.5 (S) (V)	6.6 (S) (V)	6.0 (S) (V)	6.2 (S) (V)	6.4 (S)
SU-45	7.1 (HS) (V)	6.5 (S) (V)	6.6 (S) (V)	5.2 (S) (V)	5.3 (S) (V)	6.1 (S)
Maldandi	7.3 (HS) (V)	5.8 (S) (V)	6.4 (S) (V)	5.5 (S) (V)	5.6 (S) (V)	6.1 (S)
CSV – 28	5.2 (S) (V)	3.4 (MR) (A)	4.5 (MR) (A)	2.7 (R) (A)	3.2 (MR) (A)	3.8 (MR)
PC-1080	3.1 (MR) (A)	2.4 (R) (A)	2.8 (R) (A)	2.4 (R) (A)	2.6 (R) (A)	2.6 (R)
PJ-1430	2.9 (R) (A)	2.4 (R) (A)	2.6 (R) (A)	2.0 (R) (A)	2.3 (R) (A)	2.4 (R)
CSV-23	3.8 (MR) (A)	3.3 (MR) (A)	3.7 (MR) (A)	2.7 (R) (A)	3.1 (MR) (A)	3.3 (MR)
IS-1077	5.3 (S) (V)	4.7 (MR) (A)	5.1 (S) (V)	3.9 (MR) (A)	4.8 (MR) (A)	4.7 (MR)
Mean	5.2 (V)	4.4 (A)	4.8 (A)	3.8 (A)	4.2 (A)	
		SEm±	CD (*P*=0.05)			
Host line		0.04	0.11			
Isolates		0.02	0.06			
Host line x isolate		0.09	0.26			

*Mean of three replication, 20 plants per replication, Disease score; 0.1-1 Highly Resistant (HR), 1.1-3 Resistant (R); 3.1-5 Moderately Resistant (MR), 5.1-7 Susceptible (S), 7.1-9 Highly Susceptible (HS) A-Avirulent and V- Virulent, * (Ambient weather condition 2014: Temperature Mini-14.6 ºC, Max.38.5 ºC. Relative Humidity, Min. 35.0%, Max. 98.0%)
(Ambient weather condition 2015: Temperature Mini-18.2ºC, Max.36.0ºC. Relative Humidity, Min. 36.0%, Max. 97.0%)

Table 5: Latent period in hours of five isolates of *C. graminicola* on sorghum lines (*Kharif* 2014 and 2015)

Host Line	Isolate (Latent period in hr*) 2014						Isolate (Latent period in hr*) 2015					
	Udr Cg-01	Chr Cg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	Mean	Udr Cg-01	Chr Cg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	Mean
IS 8354	108	114	114	120	114	114	108	114	114	120	120	115.2
IS 18442	96	102	102	108	102	102	96	102	102	108	108	103.2
IS 2312	78	78	78	90	84	81.6	72	84	78	90	84	81.6
H-112	90	96	96	108	102	98.4	90	96	96	108	102	98.4
IRAT 204	108	114	114	120	120	115.2	108	120	108	120	120	115.2
K local	72	78	72	84	84	78	78	78	78	84	78	79.2
CSV – 21	114	114	114	120	120	116.4	114	120	114	120	114	116.4
Rampur local	72	78	72	84	84	79.2	78	78	78	84	84	80.4
SSG 59-3	108	114	108	114	114	111.6	108	114	108	120	114	112.8
Pant chari 5	96	96	102	108	108	102	102	96	102	108	108	103.2
Raj chari 1	78	78	78	84	84	80.4	96	108	102	114	108	105.6
SU-45	78	90	84	96	90	87.6	84	90	90	102	96	92.4
Maldandi	78	96	84	96	96	90	84	96	84	96	96	91.2
CSV – 28	96	108	96	114	108	104.4	96	108	96	114	108	104.4
PC-1080	96	108	108	114	114	108	108	114	114	120	114	114
PJ-1430	108	114	108	120	114	112.8	108	114	114	120	114	114
CSV-23	108	114	108	114	114	111.6	108	114	114	120	114	114
IS-1077	96	102	96	108	102	100.8	96	102	96	114	108	103.2
Mean	93.3	99.7	96.7	105.7	103.0		96.3	102.7	99.3	109.0	105.7	
		S.Em±	CD (*P*=0.05)					S.Em±	CD (*P*=0.05)			
Host line		1.11	3.11					1.12	3.12			
Isolates		0.59	1.64					0.59	1.65			
Host line x isolate		2.49	6.95					2.50	6.98			

* Mean of three replications; (Ambient weather condition 2014: Temperature Mini-14.6 ºC, Max.38.5 ºC. Relative Humidity, Min. 35.0%, Max. 98.0%)
(Ambient weather condition 2015: Temperature Mini-18.2ºC, Max.36.0ºC. Relative Humidity, Min. 36.0%, Max. 97.0%)

Table 6: Disease severity and reaction of sorghum lines to five isolates of *C. graminicola* (*Kharif* 2014 and 2015)

Host Line	Disease severity 2014 (Disease score 1-9 scale) *						Disease severity 2015 (Disease score 1-9 scale) *					
	Udr Cg-01	Chr Cg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	Mean	Udr Cg-01	Chr Cg-02	Bhl Cg-03	Rjs Cg-04	Kot Cg-05	Mean
IS 8354	3.2 (MR)	2.6 (R)	2.9 (R)	2 (R)	2.5 (R)	2.6 (R)	3.0 (R)	2.4 (R)	2.8 (R)	1.7 (R)	2.4 (R)	2.5 (R)
IS 18442	5.4 (S)	4.8 MR)	4.9 (MR)	4.1(MR)	4.5(MR)	4.7(MR)	5.3 (S)	4.4 (MR)	4.7 (MR)	4.1 (MR)	4.3 (MR)	4.6 (MR)
IS 2312	7.8 (HS)	6.6 (S)	6.9 (S)	5.9 (S)	6.2 (S)	6.7 (S)	7.5 (HS)	6.4 (S)	6.6 (S)	5.9 (S)	6.1 (S)	6.5 (S)
H-112	6.1 (S)	5.4 (S)	5.4 (S)	4.4(MR)	4.5(MR)	5.2 (S)	6 (S)	5.2 (S)	5.3 (S)	4.3 (MR)	4.5 (MR)	5.1 (S)
IRAT 204	2.6 (R)	2.1 (R)	2.3 (R)	1.8 (R)	2.3 (R)	2.2 (R)	2.3 (R)	1.8 (R)	2.2 (R)	1.6 (R)	1.8 (R)	1.9 (R)
K local	8.5 (HS)	7.8 (HS)	8.3 (HS)	6.6 (S)	7.3 (HS)	7.7 (HS)	8.4 (HS)	7.6 (HS)	8.1 (HS)	6.5 (S)	7.3 (HS)	7.6 (HS)
CSV – 21	2.2 (R)	1.7 (R)	2.1 (R)	1.6 (R)	1.7 (R)	1.9 (R)	2.1 (R)	1.5 (R)	1.9 (R)	1.4 (R)	1.4 (R)	1.7 (R)
Rampur local	8.0 (HS)	7.0 (S)	7.4 (HS)	6.6 (S)	6.8 (S)	7.2 (HS)	7.8 (HS)	7.0 (S)	7.4 (S)	6.1 (S)	6.6 (S)	7.1 (S)
SSG 59-3	3.4(MR)	2.7 (R)	3.1 (MR)	2.5 (R)	2.5 (R)	2.8 (R)	3.1 (MR)	2.6 (R)	2.9 (R)	2.1 (R)	2.4 (R)	2.6 (R)
Pant chari 5	5.5 (S)	5 (MR)	5.4 (S)	4.0(MR)	4.4(MR)	4.9(MR)	4.9 (MR)	4.4 (MR)	4.8 (MR)	3.9 (MR)	4.2 (MR)	4.4 (MR)
Raj chari 1	6.9 (S)	6.7 (S)	6.7 (S)	6.2 (S)	6.4 (S)	6.6 (S)	6.7 (S)	6.3 (S)	6.4 (S)	5.8 (S)	5.9 (S)	6.2 (S)
SU-45	7.2 (HS)	6.5 (S)	6.7 (S)	5.2 (S)	5.4 (S)	6.2 (S)	6.9 (S)	6.4 (S)	6.5 (S)	5.1 (S)	5.2 (S)	6.0 (S)
Maldandi	7.4 (HS)	5.9 (S)	6.9 (S)	5.7 (S)	5.6 (S)	6.3 (S)	7.2 (HS)	5.7 (S)	5.8 (S)	5.3 (S)	5.5 (S)	5.9 (S)
CSV – 28	5.4 (S)	3.6 MR)	4.6 (MR)	2.9 (R)	3.5(MR)	4. (MR)	4.9 (MR)	3.2 (MR)	4.4 (MR)	2.5 (R)	2.9 (R)	3.6 (MR)
PC-1080	3.1 MR)	2.5 (R)	2.8 (R)	2.3 (R)	2.5 (R)	2.6 (R)	3 (R)	2.3 (R)	2.7 (R)	2.4 (R)	2.6 (R)	2.6 (R)
PJ-1430	2.9 (R)	2.5 (R)	2.6 (R)	2.2 (R)	2.5 (R)	2.5 (R)	2.8 (R)	2.3 (R)	2.5 (R)	1.8 (R)	2.1 (R)	2.3 (R)
CSV-23	3.8 MR)	3.4 MR)	3.8 (MR)	2.8 (R)	3.1(MR)	3.4(MR)	3.7 (MR)	3.2 (MR)	3.6 (MR)	2.5 (R)	3 (R)	3.2 (MR)
IS-1077	5.4 (S)	4.9(MR)	5.3 (S)	4.1(MR)	4.9(MR)	4.9(MR)	5.1 (S)	4.4 (MR)	4.8 (MR)	3.7 (MR)	4.6 (MR)	4.5 (MR)
Mean	5.27 (V)	4.54 (A)	4.89 (A)	3.94 (A)	4.26 (A)		5.04	4.28	4.63	3.71	4.04	
		S.Em±	CD (*P*=0.05)					S.Em±	CD (*P*=0.05)			
Host line		0.06	0.16					0.06	0.16			
Isolates		0.06	0.16					0.03	0.09			
Host line x isolate		0.03	0.09					0.13	0.36			

Mean of three replication, 20 plants per replication, Disease score; 0.1-1.0 Highly Resistant (HR), 1.1-3.0 Resistant (R); 3.1-5.0 Moderately Resistant (MR), 5.1-7.0 Susceptible (S), 7.1-9.0 Highly Susceptible (HS)

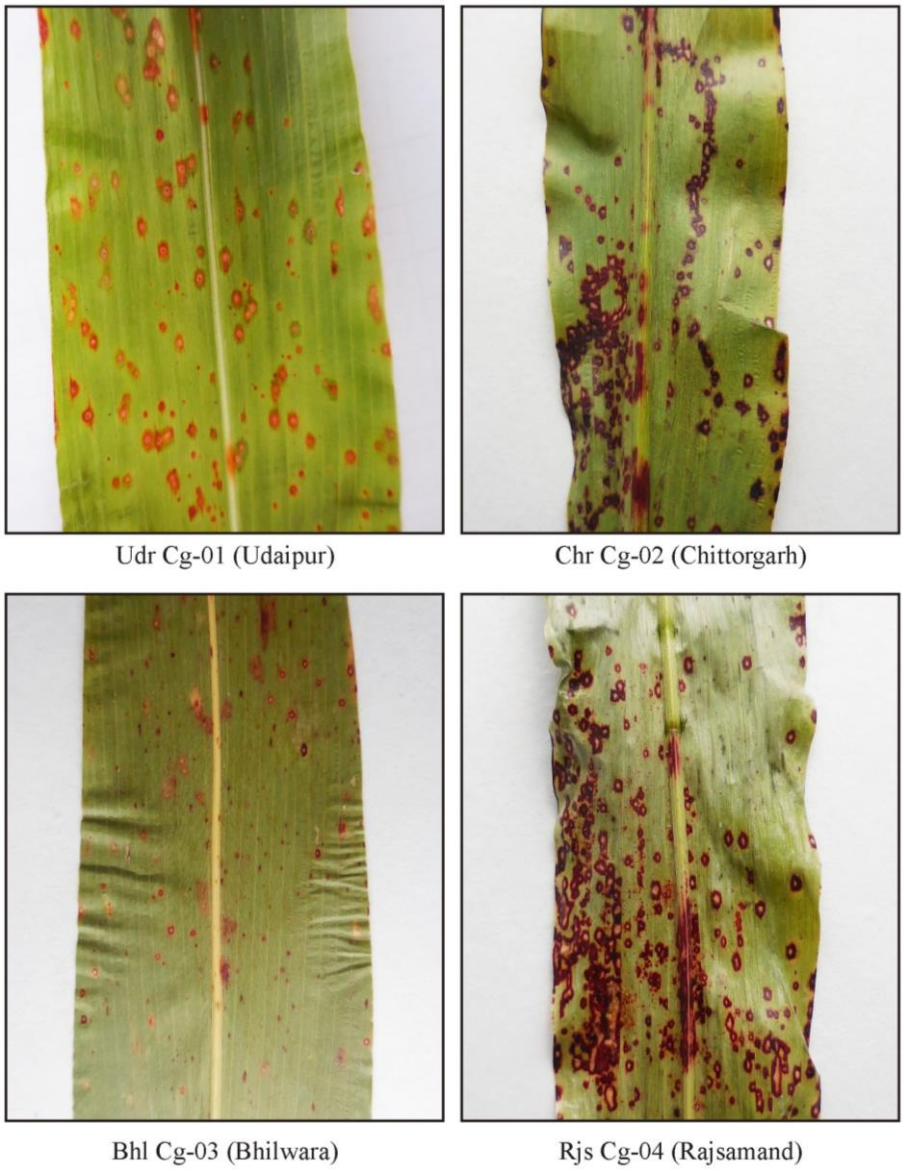


Plate 1: Variable symptoms on sorghum caused by different isolates of *C. graminicola*

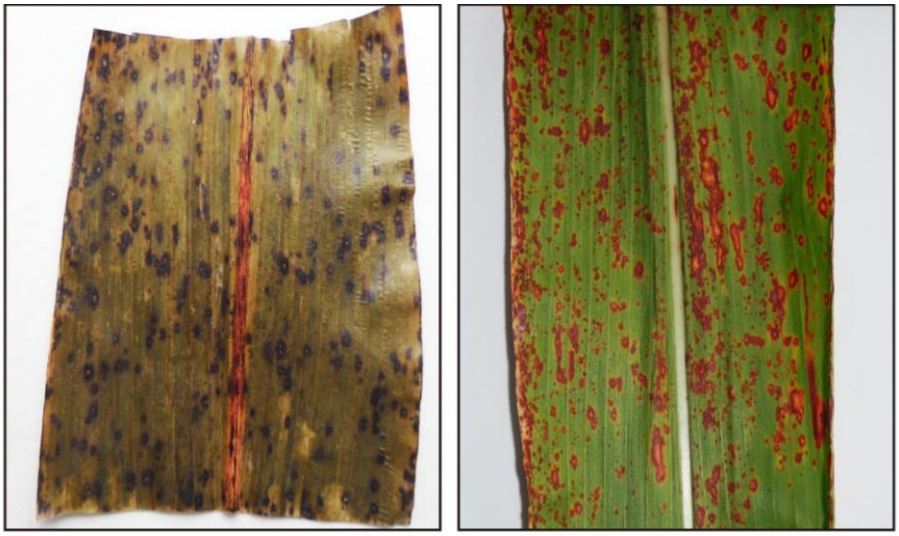


Plate 2: Variable symptoms on sorghum caused by different isolates of *C. graminicola*

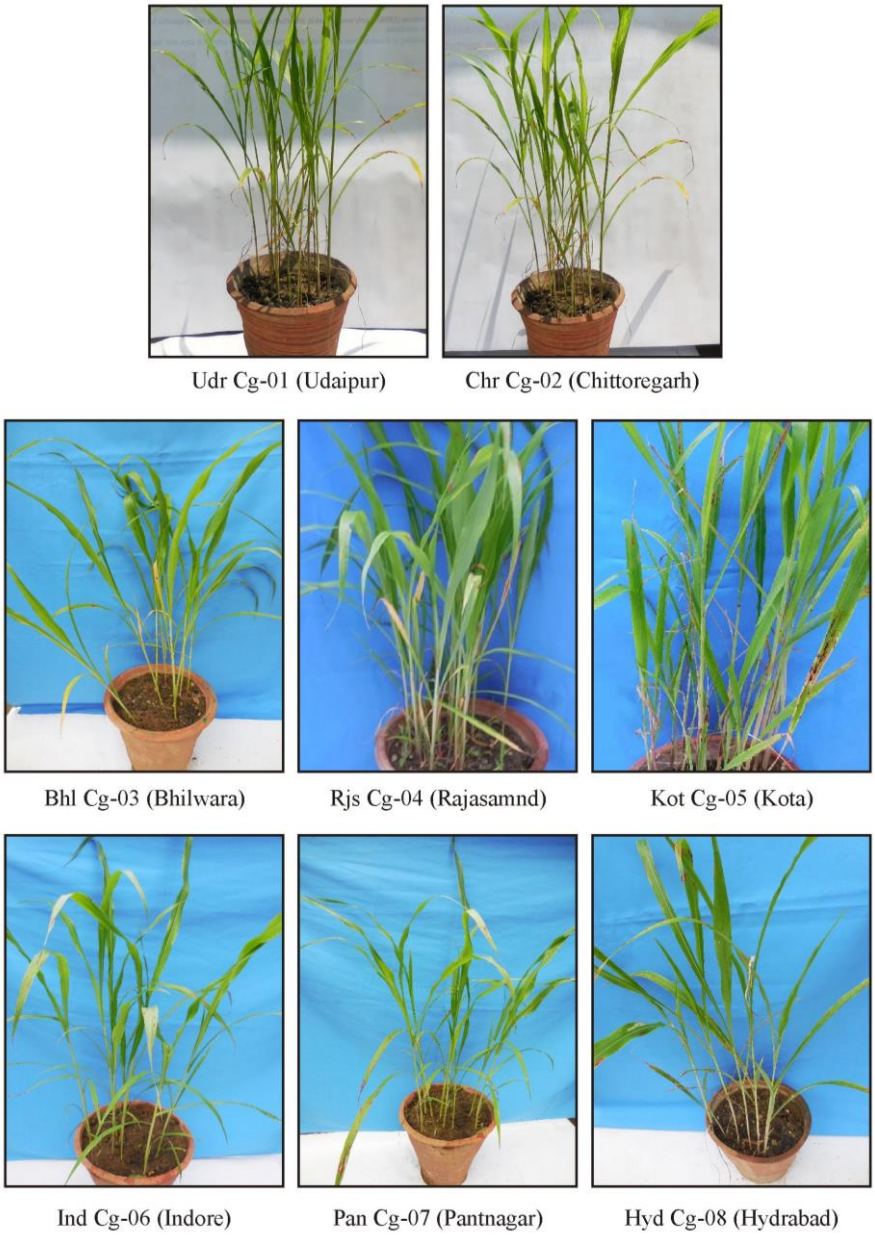
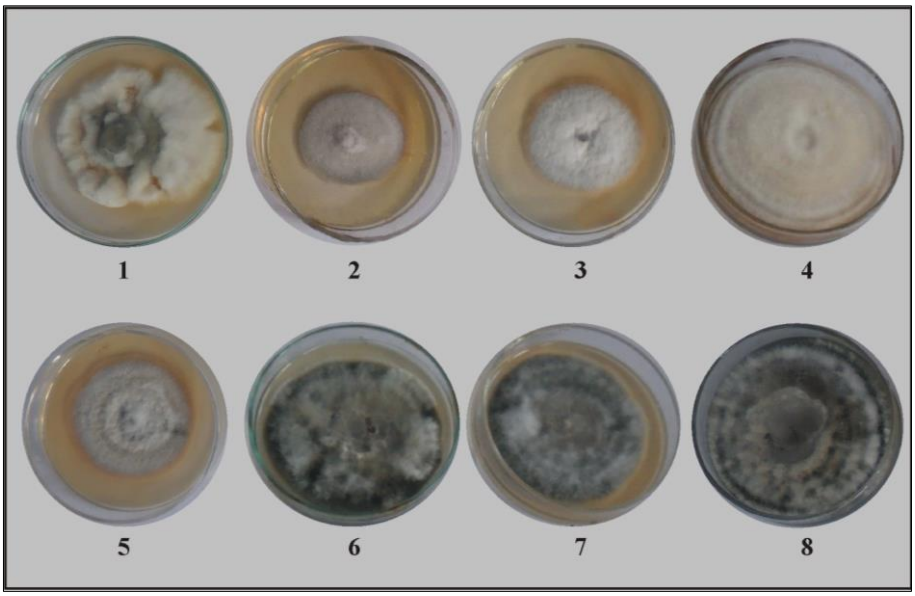


Plate 3: Pathogenicity test of the eight isolates of *C. graminicola* on sorghum cultivar K local



(1). Udr Cg-01 (Udaipur) (2). Chr Cg-02 (Chittorgarh) (3). Bhl Cg-03 (Bhilwara) (4). Rj s Cg-04 (Raj samand) (5). Kot Cg-05 (Kota) (6). Ind Cg-06 (Indore) (7). Pan Cg-07 (Pantnagar) (8). Hyd Cg-08 (Hydrabad)

Plate 4: Cultureal variability among eight isolates of C. *graminicola in vitro* (10 days after inoculation)

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