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Physiopathological studies, characterization and yield loss assessment of *Curvularia* leaf spot of maize (*Zea mays*)

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ABSTRACT

Curvularia leaf spot of maize (*Zea mays* L.) caused by *Curvularia lunata* var. aeria is one of serious foliar diseases of maize. An experimental conducted during 2016–17 at research farm of Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan found that *Curvularia* showed considerable variation in growth characteristics, colony diameter and rate of sporulation. The maximum colony diameter was 90.0 mm with 11.0 × 10^4 conidia/mm^2. The size measurement of conidia of *C. lunata*-01 was in range of 60.1–91.9 µm length and width range was 16.2–23.5 µm. Length and width of isolate *C. lunata*-02 recorded in range of 55.6–79.5 and 14.2–22.5 µm respectively. Host range distribution showed that pathogen has a wide host range in many crops and weeds under artificial inoculation. Seed transmission results showed that Local surya exhibited higher 66.66% seeds borne inoculam while Pratap makka-3 have 40.00% seed borne inoculums of pathogen. Physiopathological studies shows that 25±2°C was optimum temperature for both the isolates. Maximum mycelial growth and sporulation was found at 90% RH followed by 80% in both the isolates. The 22.29% losses in yield due to *Curvularia* leaf spot was assessed by using Le Clerg model. The present study of pathogen biology could be used by plant pathologists to develop or redesign management strategies for the maize growers.

Keywords: Characterization, *Curvularia,* Host range, Maize, Seed transmission, Yield loss

Curvularia leaf spot (CLS) of maize (*Zea mays* L.) caused by *Curvularia lunata* var. *aeria* (Batista, Lima & Vasoncelos) Ellis, is one of the most common diseases of maize. In India, the disease was observed on maize in all states during rainy and winter season (Choudhary *et al.* 2011). Earlier the disease was considered to be of minor importance but its severity is continuously increasing in Jammu and Kashmir, Himachal Pradesh, Sikkim, West Bengal, Meghalaya, Punjab, Haryana, Tripura, Assam, Rajasthan, Utter Pradesh, Bihar, Madhya Pradesh, Maharastra, Andhra Pradesh, Karnataka and Tamil Nadu. This disease causes the reduction in yield both directly and indirectly since the pathogen can attack both leaves and seeds. The disease has been reported to cause yield loss in maize to the tune of 20–60% in China, up to 33.3% in Nigeria and 21–23% in India (Dai *et al.* 1998). *Curvularia lunata* causes leaf spots which usually appear as small light brownish circular or ovoid, surrounded by brown ring. Lesions may vary in size from >1 to 3 mm in diameter in the circular type. In advance stage of infection, these small spots cover the entire leaf area either by coalescing or increasing in number. Ultimately the leaf gives blighted appearance and dies before senescence. It may attack any part of plant at any stage of growth due to seed borne inoculum. The disease spreads into newer areas through infected seeds. The secondary infection in the standing crop is caused by air-borne conidia in the favorable weather condition in field (Rathore *et al.* 2005). It is, drastically prevalent in areas where climate is warm and humid at temperature range of 28–32°C. Looking to the increasing importance and severity of Curvularia leaf spot of maize in India, the present investigation were undertaken to study mode of transmission, host range distribution, pathogen characterization and yield loss assessment.

MATERIALS AND METHODS

Sample collection and isolation: Leaf sample infected with *Curvularia* were collected, labelled and brought to the laboratory for isolations, purification and identification of the pathogen to prove pathogenicity test. Isolation was carried out using small pieces showing typical lesions of the

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Curvularia leaf spot, were cut, washed in sterilized water, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 2 min, rinsed thrice in sterilized distilled water and transferred on potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28 ± 2°C for growth. Sub-cultures were made from the periphery of the mycelial growth, which appeared after 6–7 days. The cultures were incubated at 28 ± 2°C for growth and sporulation. All the pot, field and lab experiment were conducted at the research farm of Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan during 2016–17.

Pathogenicity test: Pathogenicity test of *Curvularia* spp. were separately tested by spray and inoculating in pot grown plants of maize cultivar Surya. The plants were raised in soil: FYM (3:1) mixture and surface sterilized seeds (0.1% $HgCl_2$ for 2 min) @5 per pot were sown. Mass multiplication of the culture was done for preparation of inoculum taking pure culture of different isolates which were grown on PDA for 10–12 days at 28 ± 2°C. in Petri-plates. Final concentration of the spores was maintained as 1×10^4 conidia/ml. The plants of 40 days were spray inoculated with the suspension (10 ml/plant) with a hand held automizer. The inoculated plants were kept in humid chamber for 24 h and then transferred in cage house and high humidity was maintained throughout the disease development period by frequent irrigations.

Identification of the pathogen: The sporulating cultures were identified on the basis of morphological characters of somatic and reproductive structures with the help of standard description (Ellis 1971) and were sent to Indian Type Culture Collection (ITCC), Division of Plant Pathology, ICAR-IARI, New Delhi, for authentic identification.

Host range distribution of C. lunata: To know the host range of this pathogen, plants of different species and genera were used for artificial inoculation by *Curvularia* spp. Plants were raised in pots with standard cultivation methods. A total of 34 hosts were inoculated with conidial suspension of *Curvularia* with inoculum concentration of 1×10^5 conidia/ml using haemocytometer. The viable conidial suspension was sprayed on the test crops for two times. The observations were recorded after symptoms appearance on the inoculated plants.

Mode of transmission of C. lunata: Seed transmission of *Curvularia* was done using roll towel method with four popular maize cultivars, viz. QPM 1-2, Pratap makka-3, HM-5 and Local surya. The seeds were soaked in sterilized water for 24 h. Plastic trays filled with sterilized sand keeping a lid of another tray of same size. Germination towel papers were used to keep the seeds inside the roll. 15 seeds of a variety kept in a rolled towel paper and moistened. Each rolled towel paper kept in trays for 5–7 days for germination and observations were recorded when the seeds germinated.

Pathogen characterization of C. lunata: Curvularia spp. was studied for their morphological and cultural characters, growth characters, (shape, size and color of the colony), rate of sporulation and size of conidia.

Effect of different temperatures on growth and sporulation of C. lunata: In this study, PDA was used as basal media and inoculated with 5 mm disc of 7 days old culture. The inoculated plates with three replication of each isolate were kept at different temperatures, viz. 15, 20, 25, 30, 35 and 40°C in BOD and other incubators. Observations were recorded after completion of mycelial growth in any of the treatment at 25±2°C.

Effect of different relative humidity levels on mycelial growth and sporulation of C. lunata: To observe the effect of RH on mycelia growth inoculated Petri plates containing PDA were kept in sealed desiccators at different RH levels (50, 60, 70, 80 & 90). Observations on radial growth (mm) were recorded when mycelial growth completes in any of the treatment and sporulation (by means of number of conidia produced).

Yield losses assessment: To know the extent of losses in yield due to CLS, a model suggested by Le Clerg (1971) was followed. The maize cultivar Local surya was sown in Randomized Block Design (RBD) in field. The row length was 3 m plant to plant and row to row 20 & 60 cm respectively. The crop was inoculated through spray and inoculation using conidial suspension on 40 DAS plants twice with two days interval. Inoculation was done in warm and humid condition at 5–6 pm before sunset. The yield data in protected (spray of Carbendazim 12% + Mancozeb 62% @2 g/lit of solution) and unprotected were recorded replication wise and the reduction in PDI was also calculated. The percent disease index was calculated as:

$$\text{Per cent disease index} = \frac{\text{Sum of all individual disease ratings}}{\text{Total numbers of plants assessed} \times \text{Maximum rating}} \times 100$$

Statistical Analysis: The data from above experiments were subjected to analysis for coefficient of deviation for laboratory and pot trials, completely randomized design was followed, while for field trials randomized block design means of the experiments were used to compare efficacy of treatment using ANOVA.

RESULTS AND DISCUSSION

Host range distribution of C. lunata: A total of 34 hosts were inoculated and their symptoms were recorded as + for disease development and – for no symptoms (Supplementary Table 1). It was revealed that many hosts were showing symptoms just like maize, bajra, sorghum, itch grass, barnyard grass and blady grass. Others were showed different types of symptoms on leaf surface. The spots were rectangular in shape, dark brown and redish coloured on buffalo grass, swollen finger grass, dallis grass, digiteria, elephant grass, carpet grass, dominican single grass, tear grass and lemon grass. Rice and wheat leaves were also showing symptoms when inoculated having dark brown, round and small sized spots. Some of inoculated hosts did not show the symptoms of *Curvularia* such as pigeon pea, greengram/moong, blackgram, cowpea/lobia, soybean, groundnut, sesamum, guar, black night shade,

Table 1 Cultural and morphological characterization of *C. lunata* using PDA medium

Isolate	Diameter (mm)	Colony colour	Margin	Zonation	Pigmentation	Sporulation ($\times 10^4$/mm^2 medium)	Size of conidia (µm)	
							Length	Width
C. lunata-01	85.5	Steel gray turning to black	Regular	Present	Ash coloured	9.33	70.4±8.79	60.1–91.2
C. lunata-02	90.0	Gray and velvety in centre	Irregular	Present	Ash coloured	11.00	65.4±5.91	55.6–79.5 19.4±1.50
SEm±			0.257			0.031	0.348	0.099
CD (P=0.05)			0.720			0.86	0.976	0.279

medick rottlepod, trefoil rottlepod, bhringaraj, prickly chaff flower and coffee senna.

Mode of transmission of C. lunata: The results obtained reveal (Supplementary Table 2) that seeds were having inoculums that too externally present. Maximum percentage of seeds infected found in maize variety Local surya (66.66) followed by QPM 1-2, HM-5 and Pratap Makka-3. Since the inoculum was present on the seed surface, the seed treatment will be very effective. It was noticed that seed carries the inoculums, which further disseminate through wind borne conidia and under favourable conditions that becomes severe. However, there are no reports regarding its exact location on seed where it sits and survive until the next season crop. There are chances of its survival in the debris as well as on same alternate or collateral host. This needs further careful and sincere investigation to come out with conclusion. These findings also are supported by Akonda *et al.* (2015).

Characterization of C. lunata: The observations were recorded for *C. lunata*-01 isolates on PDA showed diameter of growth 85.5 mm, colony colour was steel gray turning to black, margin- regular, topography raised, zonation present, pigmentation ash coloured and sporulation 9.33 × 10^4/mm^2 medium. In *C. lunata*-02 diameter of growth -90.0 mm, colony colour gray and velvety in centre, margin-irregular, topography raised, zonatin present, pigmentation ash coloured and sporulation 11.0 × 10^4/mm^2 medium. The conidia of *Curvularia* had three septate and second septa was responsible for curvature. These middle septa are generally bigger in size with minor variation among the isolates. The conidial morphology studies resulted in size variations (Table 1). Bigger size of conidia was observed in *Curvularia lunata*-01 then *C. lunata*-02, although with little difference. Both the cultures were fast growing with gray colour. Conidia in both the cultures were three septate and true to type to the genus *Curvularia*. The morphological features were found similar to that of Somal (1975) and Choudhary *et al.* (2011) but there were some differences in the morphology of conidia. There was remarkable difference in width and length of conidia which was the reason to designate them as *C. lunata*-01 and *C. lunata*-02. There is no reference available in relation to the variability among the isolates and this kind of work may be taken up in the future using molecular markers to know the variability and will also help in screening various fungicides to control this pathogen.

Table 2 Effect of different temperature on growth and sporulation of *C. lunata* on PDA medium

Temperature (±2°C)	*C. lunata*-01		*C. lunata*-02	
	Mycelial growth in diameter (mm)*	Sporulation	Mycelial growth in diameter (mm)*	Sporulation
15	26.00	+	28.00	+
20	50.00	++	52.00	++
25	89.00	+++	89.50	+++
30	87.00	+++	88.66	+++
35	70.00	++	74.66	++
40	30.00	+	31.66	+
SEm±	1.409		1.700	
CD (P=0.05)	4.341		5.240	

+ = Poor, ++ = Moderate, +++ =Good, *Mean of three replications.

Effect of different temperatures on growth and sporulation of C. lunata: The results shows that maximum mycelial growth and sporulation was found at 25±2°C. (89.00 and 89.50 mm) followed by 30°C and 35°C (Table 2). Least growth and sporulation were recorded at 15°C and 40°C. Moderate growth and sporulation was found 20°C. Therefore, it was concluded that both the isolates could grow and sporulate best at 25°C. The data were found statistically significant with CD at 5% in *Curvularia lunata*-01 as 4.341 and *C. lunata*-02 as 5.240 (Table 2). It has been also reported in the field practically that when temperature is around 25°C and RH is remaining 90% for some time. The disease symptoms progressed very fast and if this remains for longer period the disease severity is maximum (Olufolaji 1986). However, temperature and RH are necessary for crop growth but at the same time fungus also enjoys this appropriate climatic set-up by giving more sporulation and dissemination. However, this kind of experimentation can be taken up in field, which will require certain precise equipments.

Effect of different relative humidity levels on mycelial growth and sporulation of C. lunata: It was noticed that 90% RH is the best for mycelial growth (Curvularia lunata-01 88.03 and *C. lunata*-02 90.00) as well as sporulation (+++) followed by 80% (*Curvularia lunata*-01 79.66 and

Table 3 Effect of different levels of relative humidity on growth and sporulation of *C. lunata* on PDA

Relative Humidity (%)	*C. lunata*-01		*C. lunata*-02	
	Mycelial growth in diameter (mm)*	Sporulation	Mycelial growth in diameter (mm)*	Sporulation
50	60.66	+	62.33	+
60	70.66	+	67.00	+
70	74.33	++	74.66	++
80	79.66	++	80.33	++
90	88.33	+++	90.00	+++
SEm±	1.437		2.145	
CD (P=0.05)	4.527		6.760	

C. lunata-02 80.33) sporulation (++); 70% (*Curvularia lunata*-01 74.33 and *C. lunata*-02 74.66) sporulation (++); 60% (*C. lunata*-01 70.66 and *C. lunata*-02 67.00) sporulation (+) and 50% (*C. lunata*-01 60.66 and *C. lunata*-02 62.33) sporulation (+) (Table 3). This has been observed practically during crop season also, when cloudy weather is there the infection rate is high. If rains occur two days after inoculation and RH above 80% prevails for a week, the disease progress will be very high. Foliar pathogens are affected by two important physical factors, viz. temperatures and relative humidity (RH) which was studied by taking different sets of temperatures and relative humidity ranging from 15–40°C and 50–90% respectively. Optimum temperature was found to be 25±2°C with maximum mycelial growth and minimum in 15°C. Similarly maximum growth and sporulation was observed in 90% RH and minimum in 50% RH. The findings are also supported by Guo *et al.* (2003) and Olufolaji (1986).

Yield loss assessment: The experiment was conducted in accordance with Le Clerg model taking 10 replications of protected and unprotected. Yield data as well as PDI was recorded in protected (T_1) and unprotected (T_2) (Supplementary Table 3). The calculated loss in yield was 22.29% and per cent reduction in PDI was also significant. The data are statistically significant with CD at 5% PDI, Protected (0.190) and Unprotected (0.300). All the diseases cause reduction in the yield which can only be perfectly assessed by certain scientific method (Le Clerg 1971). Similar results were also reported by Ding Fa *et al.* (1999). The outcome of this experiment shows that there is reduction of 22.29% in grain yield over unprotected control. This loss is huge when we look into the total grain yield corresponding to area under cultivation in the country. Since the crop is very important in terms of food and poultry feed and large sector of industries are raw maize depended. If this loss can be minimized this will ultimately an increase in the production.

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