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Distribution and Diversity of the Sorghum Sugary Disease Pathogens in India

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Abstract

til 1991, *Claviceps sorghi* was considered the only fect stage of *Sphacelia sorghi*, the causal agent of the gary disease of sorghum in India. The presence of aviceps *africana*, a distinct pathogen and another per- t stage of *S. sorghi*, was reported during the 1990s in rica, Australia, and North and South America. In India, occurrence of *C. africana* was confirmed at a few ations in 1999 and 2000. In this chapter, we describe geographical distribution and diversity of the sugary ease pathogens in India based on an extensive survey nine states and examination of infected panicles con- ning honeydew and sclerotia/sphacelia from seven tes. The pathogen was isolated from these specimens d characterized using sphacelial, conidial, and sclero- l morphology; secondary conidia production; colony rphology and growth rate; and molecular markers. sed on these studies, *C. africana* is widespread in ven sorghum-growing states of India and is currently dominant sugary disease pathogen of sorghum in In- . *Claviceps sorghi* is now restricted to a few locations the states of Andhra Pradesh and Maharashtra.

Introduction

got, or sugary disease, of sorghum is a serious disease t affects the production of F_1 hybrid seed. Sugary dis- e is particularly severe in male sterile lines (A-lines), her when nonsynchronous flowering of A-line and re- rer lines (R-lines) occurs, or when adverse environ- ntal conditions result in a lack of viable pollen and de- ed seed set. In India, losses of 10-80% have been re- ted in hybrid seed production fields (1). Three species ve been reported to cause sugary disease on sorghum different parts of the world. These species are *Claviceps sorghi* Kulkarni, Seshadri and Hegde in India (5), *Claviceps africana* Frederickson, Mantle and de Milliano in Zimbabwe (4), and *Claviceps sorghicola* Tsukiboshi, Shimanuki and Uematsu in Japan (15). Recently, the occurrence of *Claviceps africana* was independently confirmed in India following molecular (10) and biochemical analyses (3). The imperfect stages of both *C. sorghi* and *C. africana* are named *Sphacelia sorghi* McRae (6).

Although both *C. sorghi* and *C. africana* have been reported in India, the distribution and relative abundance of these two pathogens in different sorghum growing areas is unknown. In this chapter, we briefly review the Indian literature on sugary disease of sorghum and summarize the current distribution and diversity of sugary disease pathogens in India. Our current understanding is based on studies carried out at ICRISAT during 1999-2000 to evaluate the diversity of sugary disease patho-

TABLE 12-1. Distribution of *Claviceps sorghi* and *Claviceps africana* cultures isolated from sugary disease affected sorghum panicles collected from different states in India.

State	Number of Locations	Number of Collections	Number of Cultures	
			C. africana	*C. sorghi*
Andhra Pradesh	18	29	25	4
Karnataka	21	29	23	0
Maharashtra	11	13	10	1
Rajasthan	1	3	3	0
Tamil Nadu	4	5	5	0
Uttar Pradesh	7	10	10	0
Gujarat	1	8	8	0
Total	63	97	84	5

gens in different sorghum-growing areas. We carried out disease surveys in eight states in India, recorded incidence and severity of the disease in different areas, collected infected plant materials, cultured the pathogens from field collections, established their pathogenicity, and characterized pathogen isolates using morphological, cultural, pathogenicity, and molecular features.

Disease Distribution

Sugary disease was first recorded on sorghum in 1917 after McRae (6) observed the sphacelial stage in the erstwhile Mysore state in India in 1915. Since then, the disease has been reported in other Indian states such as Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh and Delhi, and Haryana. The sclerotial stage of the pathogen was first recorded in Koilkuntla, Andhra Pradesh (12). The disease is endemic in Maharashtra (Vidarbha and Ahmednagar regions), Karnataka, Andhra Pradesh, and Tamil Nadu. During 1999-2000, we conducted disease surveys in Delhi, Haryana, Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, and Gujarat. In these surveys, we found no sugary disease in Haryana and Delhi in 1999, and the disease severity was low in the Bundelkhand area of Uttar Pradesh due to dry conditions that prevailed during post-flowering period of sorghum in these areas. However, severe sugary disease was recorded in major sorghum growing areas of Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Rajasthan, and Gujarat. In September 1999, a severe epidemic occurred in the Rampur and Moradabad districts of Uttar Pradesh, in which almost all of the spikelets of late-flowering plants were infected. In October 1999, towards the end of rainy season, an epidemic occurred in the Machinenapally village of Mahboobnagar district of Andhra Pradesh. In September 2000, several other *mandals* (particularly Kalwakurthy *mandal*) adjoining Machinenapally village also were severely affected. These three epidemics affected local land races of sorghum and led to a total failure of grain harvest.

TABLE 12-2. Cultural characteristics of *Claviceps sorghi* and *Claviceps africana* on T_2 agar medium.[a]

Characters	*C. sorghi*	*C. africana*
Growth rate (mm/day)	0.12 ± 0.005 (0.11-0.12)[b]	0.06 ± 0.014 (0.03-0.09)
Maximum radial growth (cm)	3.35 ± 0.11 (3.25-3.52)	1.71 ± 0.42 (1.12-2.93)
Colony type	Puffy or cottony, submerged with diffused margin	Compact, raised, margin distinct
Puckering nature	None	Low to high
Sporulation	Present	Absent

[a]Average of data from five isolates of *C. sorghi* and 84 isolates of *C. africana*.
[b]Values are mean ± standard deviation, with range in parentheses.

Pathogen Distribution

Until recently, *C. sorghi* was considered the only sugary disease pathogen in India, and *C. africana* was thought to prevail in all other sorghum-growing areas. However, *C. africana* was identified recently from samples collected from a few locations in India (3, 10). From the infected materials collected during the disease surveys in 1999-2000, we cultured 89 isolates and determined their cultural characters. A comparison of cultural and molecular characters (see below) led us to group the isolates putatively as either *C. sorghi* or *C. africana*. We identified *C. africana* in all of the sorghum-growing regions of Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Rajasthan, and Gujarat (Table 12-1). *Claviceps sorghi* was restricted to northern Andhra Pradesh (Adilabad district) and Maharashtra (Yavatmal district), showing that *C. africana* is now more widely distributed in India than *C. sorghi*.

Fungal Morphology

Cultural characteristics. There are several reports of cultural characters of *C. sorghi* from India (13). On T_2 medium (8), *C. sorghi* produces a white mycelium with abundant aerial hyphae. The colony growth rate is slow compared to that of the rye ergot pathogen *C. purpurea* at 26-28°C (11). On T_2 medium, the culture sporulated, in the form of honeydew secretions, 15 days after inoculation and the color of the honeydew changed from colorless to pale brown or pinkish at later stages (7).

We found that *C. sorghi* and *C. africana* vary widely with respect to the rate of colony growth, colony type, puckering of the colony surface, and sporulation on medium. The culture of *C. sorghi* was white, puffy, cottony, and submerged, with diffused margins, while *C. africana* produced a white, raised, compact colony, with distinct margin (Table 12-2). *Claviceps sorghi* sporulated with honeydew secretions at the center of colonies, but *C. africana* did not sporulate on this medium, confirming the earlier report of Bogo and Mantle (3). *Claviceps sorghi* grew faster than *C. africana* in culture (Table 12-2). A comparison of our results to those obtained previously (7, 11, 13) from India suggests that *C. sorghi* might have been the only causal agent for sugary disease of sorghum recorded in India prior to the 1990s.

Sphacelia. Sphacelia are white, rounded to egg-shaped structures present in infected sorghum florets protruding between the glumes (2). The difference in sphacelial morphology between *C. sorghi* and *C. africana* was described by Frederickson *et al.* (4). The sphacelia of *C. sorghi* are cylindrical, curved or straight, bilaterally grooved, and visible 1-2 days after honeydew exudation begins (8-10 days after inoculation). In contrast, *C. africana* has bulky, soft, white, highly convoluted, oval to spherical sphacelia that were visible 6-7 days after inoculation (4). Variations in size and shape of sphacelia also were observed in our collections. *Claviceps sorghi* had more oblong to elliptical, and larger (4.3-4.7 × 2.9-3.0 mm), sphacelia than did *C. africana*. Sphacelia of *C. africana* were elliptical to conical or sometimes spherecal, measuring 2.9-3.6 × 1.9-2.2 mm (Table 12-3).

Conidia. The fungus produces three types of single-celled, hyaline spores: oblong to oval macroconidia, spherical microconidia, and pear-shaped secondary conidia. Macroconidia of *C. sorghi* are hyaline, elliptical or ovate with smooth ends, and with distinct vacuoles measuring 8-19 × 4-6 µm (5). Frederickson *et al.* (4) describe macroconidia of *C. africana* as oblong to oval measuring 9-17 × 5-8 µm, and slightly constricted at the center, with two polar vacuoles. In our studies, the macroconidia of *C. africana* were more oblong to elliptic and measured 10.2-18 × 6.4-9 µm, whereas *C. sorghi* produced more cylindrical, slender macroconidia measuring 9-18 × 5.1-7.7 µm (Table 12-3). *C. sorghi* and *C. africana* cannot be distinguished based on the size or shape of the microconidia.

At high relative humidity, macroconidia germinate iteratively, giving rise to secondary conidia. Secondary conidiation is a common feature of *C. africana* and is occasionally observed in *C. sorghi* (1). We compared the degree of secondary conidiation in isolates of *C. sorghi* and *C. africana* at different temperature (10, 15, 20, 25, 30, and 35°C) and relative humidity (80, 85, 90, 95, and 100% RH). Isolates of *C. africana* produced 8 to 18 times more secondary conidia at 25°C than isolates of *C. sorghi*. For both pathogens, maximum secondary conidiation occurred at 25°C followed by 20°C, and at 100% RH followed by 95% RH. *C. africana* produced more secondary conidia than *C. sorghi* at all temperature and RH levels.

Sclerotia. Bandyopadhyay *et al.* (1) summarized morphological and biochemical features that have been used to differentiate sclerotia of *C. africana* from *C. sorghi*. The sclerotia of *C. africana* are hard, spherical to oval, and largely confined within the host glumes only rarely protruding for more than a few mm beyond the glumes.

TABLE 12-3. Morphological characteristics of sphacelia, conidia, and sclerotia of *Claviceps sorghi* and *C. africana*.

Structure	*C. sorghi*	*C. africana*
Sphacelia[a]		
Size (mm)	4.3-4.7 × 2.9-3.1[b]	2.9-3.6 × 1.9-2.3
Shape and color	Oblong, cylindrical to elliptical, white	Conical, elliptical to spherical, white
Macroconidia[a]		
Size (µm)	9.0-18.0 × 5.1–7.7	10.2–18.0 × 6.4-9.0
Shape and color	Cylindrical to elliptical, hyaline	Oblong to elliptical, hyaline
Microconidia[a]	Spherical, hyaline, 2.6-3.9 µm	Spherical, hyaline, 2.6-3.9 µm
Sclerotia (with sphacelial cap)[c]		
Size (mm)	8.2-9.6 × 2.0-2.3	4.1-4.4 × 1.4-1.9
Shape and color	Cylindrical to conical, light yellowish brown	Conical to spherical, light yellowish brown
Sclerotia (without sphacelial cap)[c]		
Size (mm)	3.7-3.9 × 1.8-2.0	2.2-2.4 × 1.2-1.3
Shape and color	Oblong to cylindrical, light reddish brown	Conical to spherical, dark reddish brown

[a]Summary of data obtained from two isolates of *C. sorghi* and eight isolates of *C. africana*.
[b]Values are ranges.
[c]Summary of data obtained from four isolates of *C. sorghi* and three isolates of *C. africana*.

Sclerotia of *C. sorghi* are thin, elongate, soft or hard, and protrude as much as 15 mm beyond the glumes. The alkaloid dihydroergosine is synthesized in the sclerotia of *C. africana*, but not in *C. sorghi*. Based on their size and shape, sclerotia of both *C. africana* and *C. sorghi* were found in India during the 1999-2000 field survey (Table 12-3). We did not test the sclerotia of the Indian *C. africana* strains for the presence of dihydroergosine. Collections from varieties CSH 9, JK 22, Yellow Jowar, and MSH 51 all had well-formed sclerotia, suggesting that the production of sclerotia is favored on these genotypes.

Molecular Characterization

Sclerotia of *C. africana* do not germinate easily, and characters associated with its perfect stage have been studied only once (4). Molecular markers have been used to elucidate the phylogenetic and taxonomic relationships between *Claviceps* spp. (8, 9). Differences in nucleotide sequence of the ribosomal internal transcribed spacer region 1 (ITS1) and 5.8S rDNA regions can differentiate *C. sorghi* and *C. africana* (10). Occurrence of *C. africana* at Dharwad (Karnataka state), Patancheru (Andhra Pradesh state), and Akola (Maharashtra state) has been confirmed by the analyses of amplified fragment length

polymorphism (AFLP), random amplified microsatellite (RAM) (14), and random amplified polymorphic DNA (RAPD) banding patterns (10). Research is currently in progress in the United States, Australia, and the Czech Republic to characterize the isolates collected in India during 1999-2000. Preliminary data from these molecular studies indicate that *C. sorghi* is present in Adilabad district in Andhra Pradesh and Yavatmal district in Maharashtra. Isolates collected from other locations either belong to *C. africana* or have intermediate genotypes between *C. sorghi* and *C. africana*. Isolates identified as *C. sorghi* and *C. africana* based on morphological and cultural characteristics were consistent with the identifications made by using molecular markers. The cultural characteristics of the intermediate types, as identified by molecular markers, were similar to *C. africana*. These intermediate types might be progeny of interspecific crosses between *C. africana* and *C. sorghi*.

Conclusions

Our survey confirms the presence of both *C. africana* and *C. sorghi* in India. *Claviceps africana* now dominates in all of the sorghum-growing regions in India, and the native *C. sorghi* is restricted to a few locations in Andhra Pradesh and Maharashtra. Molecular characterization of all the isolates collected will provide further information on the diversity of the sugary disease pathogens, particularly with respect to the presence of isolates that are intermediate between *C. africana* and *C. sorghi*. The widespread occurrence of *C. africana*, and its apparent displacement of *C. sorghi* in India, may be explained by the differences in the production of secondary conidia, which largely determines the reproductive and dissemination potential of the two *Claviceps* species. Consequently, *C. africana* can potentially spread rapidly over a relatively large area in a relatively short time. It is not clear when *C. africana* arrived in India and began replacing *C. sorghi* as the dominant pathogen. Based on a re-view of literature, Pažoutová *et al.* (10) concluded that these changes might have begun during the late 1970s. However, the large-scale replacement of *C. sorghi* by *C. africana* as the causal organism of sugary disease in India, makes it necessary to re-evaluate sorghum ergot management options developed prior to the 1980s.

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