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# Cultural characteristics, morphology, and variation within *Claviceps africana* and *C. sorghi* from India

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## ABSTRACT

Sorghum ergot in India is caused by *Claviceps africana* and *C. sorghi*. The distributions of these two species in India is not known. Eighty-nine sorghum ergot isolates were cultured from young sphacelia obtained from male sterile sorghum plants artificially inoculated using inoculum collected in the field. Based on cultural characteristics, the isolates were separated into two groups which differed distinctly in the morphology of their sphacelia, conidia, and sclerotia. Marked differences also were observed in rates of secondary conidial production and disease spread between the groups. In combination with molecular evidence, our results confirm that the isolates placed in Group I represent *C. africana* and Group II isolates represent *C. sorghi*. *C. africana* was found to be widely distributed in all sorghum growing areas of India. The species first described as occurring in India, *C. sorghi*, appears to be restricted to a few locations in the states of Maharashtra, Andhra Pradesh, and Karnataka.

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## Introduction

Ergot or sugary disease of sorghum (*Sorghum bicolor*) caused by *Sphacelia sorghi* is a serious threat to the production of F<sub>1</sub> hybrid seeds, particularly if nicking is poor or seed set is delayed in male sterile lines. The disease, initially restricted to Asia and Africa, attained global significance in the late 1990s after causing epidemics in the Americas and Australia (Bandyopadhyay *et al.* 1998; Pažoutová *et al.* 2000). Three different *Claviceps* species are known to cause sorghum ergot in different parts of the world viz., *C. sorghi* reported from India (Kulkarni *et al.* 1976), *C. africana* initially reported from Zimbabwe (Frederickson *et al.* 1991), and *C. sorghicola* reported

from Japan (Tsukiboshi *et al.* 1999). In India, sorghum ergot has been reported since the early 1920s, in Maharashtra (Ajrekar 1926), Karnataka (Kulkarni 1942), Tamilnadu (Nath & Padwick 1941; Thomas *et al.* 1945; Ramakrishnan 1947), Andhra Pradesh (Ramakrishnan 1948; Sundaram 1969; Patil *et al.* 1968), and Delhi and Haryana (AICSIP 1969–1970). Kulkarni *et al.* (1976) observed the teleomorph of *Sphacelia sorghi* and described it as *C. sorghi*. Ergot is endemic in the Indian states of Maharashtra (Vidarbha and Ahmednagar regions), Uttar Pradesh, Karnataka, Andhra Pradesh, and Tamilnadu (Bandyopadhyay *et al.* 2002).

Like all ergot species, sorghum ergot colonizes ovaries, inhibiting normal pollination, fertilization, and production of

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seed. Instead, the ovaries are colonized by fungal hyphae that develop into spore-bearing fungal masses (sphaecelia), which later turn into hard sclerotia. An early report from India on cultural characters of the pathogen (Ajrekar 1926) describes the fungus as *S. sorghi*, which is the name applied to anamorph of both *C. sorghi* and *C. africana*. Colonies of *S. sorghi* on Kirchoff's medium are white with abundant aerial hyphae. Some Indian isolates cultured by Bogo and Mantle (1999) and putatively belonging to *C. africana* produced compact, white growth, which failed to produce spores on asparagine-sucrose-salts agar medium. *C. sorghicola* is a third ergot-causing *Claviceps* species reported from Japan, which has white to cream-coloured, velvety colonies, and a hyphal growth rate of 1 mm day<sup>-1</sup> at 25 °C (Tsukiboshi et al. 1999).

The anamorph of *C. africana* and *C. sorghi* is recognized in planta by the presence of sphaecelia, macroconidia, microconidia, and secondary conidia. The teleomorph phase of the pathogen begins with the production of sclerotia which upon germination give rise to ascomata, which bear ascospores. The three distinct *Claviceps* species differ in the morphology of their sphaecelia, conidia, sclerotia, and ascomata (Bandyopadhyay et al. 1996; Frederickson et al. 1991; Tsukiboshi et al. 1999). The occurrence of *C. africana* in India, in addition to *C. sorghi*, which is apparently confined to the Indian sub continent (Frederickson et al. 1991), was confirmed by Bogo and Mantle (1999) on the basis of alkaloid analyses of sclerotia of Indian isolates via gas chromatography and mass spectrometry.

Tooley et al. (2000, 2002) reconfirmed the presence of *C. africana* in India, by means of random amplified microsatellite (RAM) and AFLP analyses, using sorghum ergot isolates collected from several locations in India. Pažoutová et al. (2000) confirmed the occurrence of *C. africana* in Dharwad (Karnataka), Patancheru (Andhra Pradesh) and Akola (Maharashtra), India through RAPD analysis and Pažoutová and Bogo (2001) showed using RAPDs and rDNA (ITS) sequence analysis that certain Indian isolates collected in 2000 were *C. sorghi*. Though there are several reports of the occurrence of both *C. africana* and *C. sorghi* in India, very little knowledge exist of their distribution or cultural characteristics such as comparative morphology of sphaecelia, conidia, and sclerotia. It has been theorized (Bandyopadhyay et al. 2002; Pažoutová et al. 2000) that *C. africana* replaced *C. sorghi* as the primary ergot pathogen in India due to its increased ability to form airborne secondary conidia on the surface of abundant honeydew containing primary conidia. This ability would serve as a selective advantage during ergot epidemics, which could gradually result in the predominance of *C. africana*. In addition, *C. sorghicola*, which was reported from Japan (Tsukiboshi et al. 1999), has never been reported from India. However, its presence in India would be expected given patterns of global movement of sorghum seed. Similarity among Indian, Australian and Japanese isolates of *C. africana* for AFLP and RAPD markers were theorized to exist in part because of such patterns of seed movement (Komolung et al. 2002; Pažoutová et al. 2000; Tooley et al. 2002). To complete this study we conducted an extensive survey of sorghum growing regions in India. Our goals were to identify the *Claviceps* species causing sorghum ergot in India, determine the distribution of the species, describe the range of cultural and morphological features possessed by each

species, and compare production of secondary conidia and rates of disease spread. Many of the isolates described in this study have been further characterized using AFLPs and analysis of selected DNA sequences in a companion study (Tooley et al. 2006).

## Materials and methods

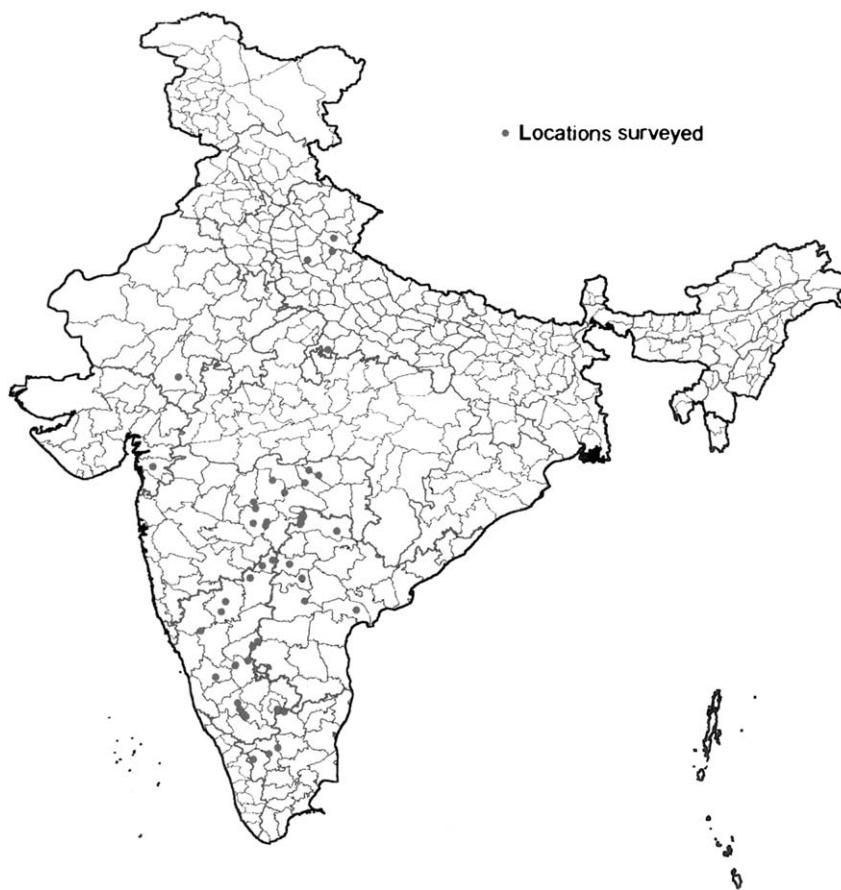
### Collection of isolates

Sorghum ergot surveys were conducted from October 1999 to February 2000 to determine the incidence and severity of the disease in major sorghum-growing Indian states (Navi et al. 2002a,b). This study is based on 89 ergot collections from 59 locations in seven sorghum-growing states of India (Fig 1, Table 1). Individual locations were represented by up to eight collections. Disease incidence and severity at the 59 locations ranged from trace levels to 100 % (Navi et al. 2002b). Between five and 25 infected panicles with honeydew or sclerotia were collected randomly from fields during the surveys. Isolates KA58b and KA58b-1 represent separate isolates from two different sphaecelia in the same panicle collected from Jalasangi, while isolates KA61b, c, d, and e represent isolates from different panicles collected in ARS-Bidar. Latitude and longitude of the locations were measured using a hand-held global positioning system instrument (Magellan System, San Dimas, CA). In addition, collaborating scientists from the National Research Center for Sorghum (Hyderabad) and several state agricultural universities collected infected panicles from their research stations and provided samples for this study. Information on approximate geographic coordinates of these stations was gathered from maps or directly from the collaborators.

### Inoculation, isolation, and morphological characterization

Panicles were brought to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India) for analysis. Two to five subsamples, each containing up to five panicles, were established from the field-collected infected sorghum panicles at each location. One subsample was dried under shade and preserved as an herbarium specimen for future reference. The remaining subsamples were used to isolate the pathogen following rejuvenation on sorghum plants. A rachis with at least three to five infected spikelets, containing either fresh or dried honeydew exudation was excised from one infected panicle of each isolate and suspended in sterile water to release the conidia. Some specimens did not have easily identifiable honeydew. For these specimens, sphaecelia or sclerotia were carefully removed from spikelets, macerated in sterile water with a mortar and suspended in water. The inoculum suspension was filtered through two layers of cheesecloth (changed after each specimen), and adjusted to  $1 \times 10^6$  propagules ml<sup>-1</sup> using a haemocytometer.

Plants of male sterile sorghum genotype 296A were maintained in pots outdoors until the flag leaf stage, and then moved to a glasshouse. As tips of panicles began to emerge from the boot leaves, they were covered with pollination



**Fig 1 – Map of India, showing locations from which sorghum ergot was collected during the 1999–2000 season.**

bags to avoid unintentional infection. At the 50 % flowering stage, bags were briefly removed, panicles sprayed with inoculum suspension, and rebagged. At least three panicles were inoculated with each subsample. Inoculated plants were incubated for 24 h at 25 °C in a dew chamber to provide favourable conditions for infection after which plants were returned to the glasshouse for disease development.

Inoculated sorghum panicles were examined daily from 4 d postinoculation onward until the first appearance of white sphacelia between the glumes, which indicates successful infection (Bandyopadhyay et al. 1990). Spikelets with newly-formed ergot sphacelia were excised before they produced honeydew. The young sphacelia were teased out of glumes and surface-sterilized in 1 % sodium hypochlorite for 3 min followed by five rinses in sterile distilled water. They were then transferred to 9 cm diam Petri plates containing T2 sucrose-asparagine-salts agar medium (Pažoutová et al. 2000) and incubated at 25 °C. At least ten sphacelia from each subsample were plated. Cultures of *Claviceps africana* and *C. sorghi* grew from the sphacelia on T2 agar, and subcultures of each isolate are deposited at the Indian Type Culture Collection [Department of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi].

Growth and morphological characteristics of ten subcultured colonies from each isolate growing on T2 agar medium at 25 °C were recorded at 7 d intervals. The maximum colony

diameter (following 30 d incubation at 25 °C), sporulation, colour, colony type, sulcate (puckering or folding of mycelial mass) nature, and pigmentation in the culture media were recorded for each isolate. The sulcate (puckering) nature of the colonies was recorded by using an arbitrary scale representing low, medium and high degree of puckering. Pigmentation was recorded using Munsell's colour chart (Munsell Products 1973). The growth rate of colonies ( $\text{mm d}^{-1}$ ) were calculated from colony diameter measured at weekly intervals.

The ergot collections were categorized on the basis of colony type and sporulation on T2 agar medium. After categorizing the collections, at least one isolate from each state where the group was present was randomly selected to represent each group.

#### **In vivo characterization of sphacelia, conidia and sclerotia**

Sorghum panicles of male sterile line 296A were inoculated with representative isolates as described above. Infected spikelets were sampled 7 d after inoculation and sphacelia were dissected out of the glumes without damaging the sphacelial tissues. The colour, shape, and size (length and breadth after cutting the sphacelia in half longitudinally) of 25 sphacelia of each isolate were recorded. Aqueous microscope slide mounts were made with 2 d-old honeydew from infected panicles to observe shape, size, and constriction at the centre of

**Table 1 – Sources and cultural characteristics<sup>a</sup> of 89 Indian *Claviceps* isolates collected between October 1999 and February 2000**

Isolate	State, district, locality (cultivar)	Rate of growth (mm d <sup>-1</sup> )	Max. radial growth <sup>b</sup> (cm)	Colony type	Puckering nature	Pigmentation	Sporulation
NAP1	Andhra Pradesh, Ranga Reddy, Tammalonigudem (Local)	0.04	1.3	Compact	L	2.5 YR 4/5	Absent
NAP 2	Andhra Pradesh, Nalgonda, Chakalisherupally (Yellow Jowar)	0.04	1.21	Compact	M	2.5 YR 5/4	Absent
NAP3	Andhra Pradesh, Nalgonda, Injamuru (Local)	0.04	1.33	Compact	M/H	2.5 YR 3/4	Absent
NAP4	Andhra Pradesh, Adilabad, Adigam (CSH9)	0.11	3.27	Cottony	Nil	2.5 YR 6/6	Present
NAP5	Andhra Pradesh, Adilabad, Dongregaw (CSH0)	0.11	3.25	Cottony	Nil	2.5 YR 6/6	Present
NAP6	Andhra Pradesh, Adilabad, Gudihatnur (CSH9)	0.07	2.1	Compact	L	2.5 YR 5/6	Absent
NAP7	Andhra Pradesh, Adilabad, Sitagondi (CSH9)	0.12	3.33	Cottony	Nil	2.5 YR 5/4	Present
NAP8	Andhra Pradesh, Medak, Chintakunta (CSH9)	0.04	1.4	Compact	M	2.5 YR 5/6	Absent
NAP9	Andhra Pradesh, Adilabad, Kamalapuram (CSH9)	0.08	2.4	Compact	L	2.5 YR 5/5	Absent
NAP10	Andhra Pradesh, Adilabad, Mannur (CSH9)	0.05	1.64	Compact	M	2.5 YR 4/6	Absent
NAP11	Andhra Pradesh, Adilabad, Naradegonda (CSH9)	0.04	1.2	Compact	L	2.5 YR 4/5	Absent
NAP12	Andhra Pradesh, Adilabad, Kupti (CSH9)	0.04	1.32	Compact	M	2.5 YR 5/6	Absent
NAP13	Andhra Pradesh, Adilabad, Burkapalle (JK 22)	0.12	3.4	Cottony	Nil	2.5 YR 5.5/4	Present
APAU1	Andhra Pradesh, Ranga Reddy, Rajendranagar (27B)	0.05	1.52	Compact	L	2.5 YR 5/6	Absent
APAU2	Andhra Pradesh, Ranga Reddy, Rajendranagar (296 B)	0.04	1.22	Compact	L	2.5 YR 6/6	Absent
APAU3	Andhra Pradesh, Ranga Reddy, Rajendranagar (DSV 3)	0.06	1.95	Compact	L	2.5 YR 6/6	Absent
APAU4	Andhra Pradesh, Ranga Reddy, Rajendranagar (CS 3541)	0.04	1.24	Compact	L	2.5 YR 6/6	Absent
AP0	Andhra Pradesh, Medak, Patancheru (ICRISAT) (CSH9)	0.05	1.6	Compact	M/H	2.5 YR 6/6	Absent
AP12	Andhra Pradesh, Mahabubnagar, Maachinenapally (Yellow Jowar)	0.05	1.56	Compact	M	2.5 YR 4/5	Absent
AP13	Andhra Pradesh, Mahabubnagar, Maachinenapally (SSG 878)	0.06	1.9	Compact	L	2.5 YR 5/6	Absent
AP14w	Andhra Pradesh, Mahabubnagar, Maachinenapally (White Jowar)	0.06	1.73	Compact	L/M	2.5 YR 5.5/6	Absent
AP14Y	Andhra Pradesh, Mahabubnagar, Maachinenapally (Yellow Jowar)	0.05	1.5	Compact	M	2.5 YR 6/6	Absent
AP15	Andhra Pradesh, Mahabubnagar, Maachinenapally (Yellow Jowar)	0.06	1.8	Compact	L	2.5 YR 5/6	Absent
AP16	Andhra Pradesh, Mahabubnagar, Maachinenapally (Yellow Jowar)	0.07	2.12	Compact	L	2.5 YR 5/6	Absent
AP16RB	Andhra Pradesh, Nalgonda, Chicholi (Yellow Jowar)	0.06	1.8	Compact	L	2.5 YR 5/6	Absent
AP16VR	Andhra Pradesh, Guntur, Lam Farm (Local)	0.04	1.25	Compact	L	2.5 YR 4/6	Absent
AP17	Andhra Pradesh, Mahabubnagar, Maachinenapally (SSG 898)	0.05	1.6	Compact	L	2.5 YR 5/6	Absent
KA41	Karnataka, Bijapur, Muttagi (White Jowar)	0.04	1.2	Compact	L	2.5 YR 6.5/6	Absent
KA53a	Karnataka, Bijapur, Almel (Fodder sorghum)	0.07	2.16	Compact	M	2.5 YR 3/5	Absent
KA57b	Karnataka, Gulbarga, Rachnal (Hybrid sorghum)	0.06	1.75	Compact	L	2.5 YR 5/6	Absent
KA58b	Karnataka, Bidar, Jalasangi (Yellow Jowar)	0.05	1.5	Compact	M	2.5 YR 4/4	Absent
KA58b-1	Karnataka, Bidar, Jalasangi (Hybrid sorghum)	0.06	1.75	Compact	M	2.5 YR 5/6	Absent
KA61	Karnataka, Bidar, ARS-Bidar <sup>c</sup> (DKR 9501)	0.06	1.77	Compact	M	2.5 YR 5/6	Absent
KA61b	Karnataka, Bidar, ARS-Bidar (SVD 9601)	0.08	2.37	Compact	M	2.5 YR 5/6	Absent
KA61c	Karnataka, Bidar, ARS-Bidar (DSV 2)	0.04	1.35	Compact	L	2.5 YR 3/5	Absent
KA61d	Karnataka, Bidar, ARS-Bidar (SHD 9704)	0.06	1.75	Compact	M	2.5 YR 4/6	Absent
KA61e	Karnataka, Bidar, ARS-Bidar (SPV 1333)	0.08	2.53	Compact	L	2.5 YR 4/6	Absent

(continued on next page)

Table 1 – (continued)

Isolate	State, district, locality (cultivar)	Rate of growth (mm d <sup>-1</sup> )	Max. radial growth <sup>b</sup> (cm)	Colony type	Puckering nature	Pigmentation	Sporulation
SK1	Karnataka, Mandya, Kbettali (Popcorn)	0.06	1.9	Compact	L	2.5 YR 5/6	Absent
SK2	Karnataka, Mandya, Chinakurali (Yellow Jowar)	0.05	1.52	Compact	M/H	2.5 YR 3/2	Absent
SK3	Karnataka, Mandya, Thendekere (Local)	0.04	1.25	Compact	L	2.5 YR 5/6	Absent
SK5	Karnataka, Mandya, Laxmipura (Ogarujola)	0.05	1.4	Compact	L/H	2.5 YR 4/4	Absent
SK7	Karnataka, Hassan, Darsigatta (Ogarujola)	0.08	2.43	Compact	L/M	2.5 YR 4/6	Absent
SK12	Karnataka, Chitradurga, Hirebennur (CSH 5)	0.05	1.52	Compact	M	2.5 YR 3/4	Absent
SK13	Karnataka, Chitradurga, Obavvanagathi halli (Local)	0.05	1.6	Compact	M/H	2.5 YR 3/3	Absent
SK16	Karnataka, Chitradurga, Talaku (MSH 51)	0.06	1.9	Compact	M	2.5 YR 4/5	Absent
SK17	Karnataka, Chitradurga, Bommadevanahalli (MSH 51)	0.05	1.16	Compact	L	2.5 YR 6/6	Absent
SK-5-21	Karnataka, Bangalore, Neralur (Fodder sorghum)	0.05	1.43	Compact	L	2.5 YR 4/6	Absent
SK-14-23	Karnataka, Shimoga, Hasalli (Ogarujola)	0.05	1.65	Compact	L	2.5 YR 5/6	Absent
SK-20-24	Karnataka, Chitradurga, Talaku (MSH 51)	0.03	1.12	Compact	L	2.5 YR 4/6	Absent
SK-22-24	Karnataka, Bellary, Amarapura (GK 52)	0.04	1.42	Compact	M	2.5 YR 4/6	Absent
UASD1	Karnataka, Dharwad, Agricultural College (CSH 13)	0.09	2.93	Compact	L	2.5 YR 5/6	Absent
AK1	Maharashtra, Akola, Akola (unknown cultivar)	0.04	1.4	Compact	L	2.5 YR 5/6	Absent
MH67	Maharashtra, Akola, Risode (CSH 9)	0.05	1.4	Compact	M	2.5 YR 4/6	Absent
MH70	Maharashtra, Akola, Mana (Nilva local)	0.07	2.18	Compact	M	2.5 YR 6/6	Absent
MH71	Maharashtra, Nagpur, Chembeli (Mottura)	0.06	1.73	Compact	L	2.5 YR 5/4	Absent
MH72	Maharashtra, Nagpur, Mahagaon (Kaderu local)	0.05	1.61	Compact	L	2.5 YR 5/4	Absent
MH73	Maharashtra, Wardha, Salod (Pandharpuri)	0.05	1.43	Compact	L	2.5 YR 5/6	Absent
MH74	Maharashtra, Yavatmal, Bori (CSH 14)	0.12	3.52	Cottony	Nil	2.5 YR 4/4	Present
MH75	Maharashtra, Nanded, Malegaon (Gulbhendi red)	0.04	1.2	Compact	L	2.5 YR 5/6	Absent
MH76	Maharashtra, Nanded, Limbgaon (Pandrapiwala)	0.05	1.42	Compact	L	2.5 YR 4/4	Absent
MH78	Maharashtra, Parbhani, SRS-Parbhani (CSH 9)	0.05	1.63	Compact	L	2.5 YR 5/6	Absent
MH79	Maharashtra, Parbhani, SRS-Parbhani (SPV 1333)	0.04	1.27	Compact	L	2.5 YR 3/4	Absent
MH80	Maharashtra, Parbhani, SRS-Parbhani (Nilwa local)	0.07	2.2	Compact	L	2.5 YR 4/6	Absent
NI1	Uttar Pradesh, Rampur, Rampur (Local)	0.07	2.24	Compact	M	2.5 YR 5/6	Absent
NI2	Uttar Pradesh, Jhansi, Mouranipur (SBPR 94004)	0.05	1.63	Compact	M/H	2.5 YR 5/6	Absent
NI3	Uttar Pradesh, Jhansi, Mouranipur (ICSA 696)	0.05	1.52	Compact	H	2.5 YR 4/4	Absent
NI4	Uttar Pradesh, Nainital, Pantnagar (ICSB 91002)	0.05	1.45	Compact	M	2.5 YR 5/4	Absent
NI5	Uttar Pradesh, Nainital, Pantnagar (2219 A)	0.05	1.4	Compact	M	2.5 YR 4/4	Absent
NI6	Uttar Pradesh, Nainital, Pantnagar (2219 A)	0.06	1.76	Compact	M	2.5 YR 4/4	Absent
NI7	Uttar Pradesh, Moradabad, Drolu (Local)	0.06	1.86	Compact	H	2.5 YR 4.5/4	Absent
NI8	Uttar Pradesh, Moradabad, Didoli/Deroli (Local)	0.06	1.9	Compact	M	2.5 YR 3/4	Absent
NI9	Uttar Pradesh, Rampur, Kunapur (Local)	0.05	1.55	Compact	H	2.5 YR 5/4	Absent
NI10	Uttar Pradesh, Moradabad, Sonali (Local)	0.07	1.97	Compact	M	2.5 YR 5/6	Absent
NI11	Rajasthan, Udaipur, RCA1 (SU 1A)	0.05	1.42	Compact	H	2.5 YR 4.5/4	Absent
NI12	Rajasthan, Udaipur, RCA2 (SU 14A)	0.04	1.28	Compact	M/H	2.5 YR 5/4	Absent
NI13	Rajasthan, Udaipur, RCA3 (SU 27A)	0.03	1.05	Compact	M	2.5 YR 5/4	Absent
TN6	Tamilnadu, Dharmapuri, Nallgankothapalli (Yellow Jowar)	0.06	1.8	Compact	M	2.5 YR 4/4	Absent
TN10	Tamilnadu, Namakkal, Muniyappan Kovil (Solan local)	0.05	1.4	Compact	L	2.5 YR 5/4	Absent
TN11	Tamilnadu, Erode, Totivalayam (Manjal jolam local)	0.05	1.6	Compact	L	2.5 YR 5/4	Absent
TN12	Tamilnadu, Coimbatore, TNAU, Millets scheme (Doddamanga jola local)	0.04	1.33	Compact	L	2.5 YR 5/4	Absent

TN13	Tamilnadu, Coimbatore, TNAU, Millets scheme (TNSH 39)	0.05	1.48	Compact	L	2.5 YR 4/6	Absent
GUJ1	Gujarat, Surat, Surat (unknown cultivar)	0.07	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ5	Gujarat, Surat, Surat (unknown cultivar)	0.07	2.25	Compact	L	2.5 YR 6/6	Absent
GUJ6	Gujarat, Surat, Surat (unknown cultivar)	0.07	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ9	Gujarat, Surat, Surat (unknown cultivar)	0.08	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ15	Gujarat, Surat, Surat (unknown cultivar)	0.08	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ20	Gujarat, Surat, Surat (unknown cultivar)	0.08	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ34	Gujarat, Surat, Surat (unknown cultivar)	0.08	2.25	Compact	L	2.5 YR 5/6	Absent
GUJ35	Gujarat, Surat, Surat (unknown cultivar)	0.08	2.25	Compact	L	2.5 YR 4/6	Absent

<sup>a</sup> The maximum colony diameter, sporulation, colour, colony type, puckering nature (folding of mycelial mass), and pigmentation in the culture media were recorded for each isolate growing on T2 agar medium (Pažoutová *et al.* 2000) at 25 °C. Growth characteristics of ten subcultured colonies from each isolate were assessed on T2 agar medium and recorded at 7 d intervals. The puckering (sulcate) nature of the colonies was recorded by using an arbitrary scale representing low, medium and high degree of puckering. Pigmentation was recorded using Munsell's colour chart (Munsell Products 1973). The least significant difference (LSD) value for radial growth ( $P = 0.05$ ) was 0.017, while that for maximum radial growth was 0.072.

<sup>b</sup> Maximum radial growth following 30 d incubation on T2 agar medium at 25 °C.

<sup>c</sup> ARS, Agricultural Research Service station at Bidar.

macroconidia. Since large numbers of microconidia are found in honeydew obtained after several days of exudation (Bandyopadhyay *et al.* 1990), size and shape of microconidia were recorded in aqueous mounts made from 30 d-old honeydew that were almost dry or thick in consistency. Fifty macroconidia and microconidia were measured for each isolate.

Sclerotium collected during the survey of sorghum-growing areas were used to assess sclerotia morphology as panicles inoculated in the greenhouse did not produce sclerotia even after incubating infected plants in hot and dry conditions as specified by Bandyopadhyay *et al.* (1990). Only those collections that contained sclerotia could be used in this study. Observations were made using sclerotia with a sphacelial cap at their tip and also after carefully removing the sphacelial cap. Sphacelial caps were removed by immersing the sclerotia in warm water and using forceps to remove the sphacelial tissues from their tips without damaging the sclerotial tissues (Frederickson *et al.* 1999). Size (length and breadth), shape, and colour of 25 sclerotia per collection were recorded.

### Production of secondary conidia

The same ten isolates used for *in vivo* characterization of sphacelia were used to study variation in secondary conidia production at different temperatures and relative humidity (RH) levels. Panicles of sorghum line 296A were inoculated as described above. The day before the expected appearance of honeydew (5 d after inoculation), plants were transferred to a growth chamber maintained at 25 °C with RH <70 % to allow formation of honeydew but suppress secondary conidia production. From these panicles, rachis containing at least six spikelets with 2 d-old honeydew were carefully excised without allowing the honeydew to drop. The basal end of the excised rachis was inserted into a 100 ml conical flask or 20 ml vial containing 2 % sucrose solution covered with Parafilm to support the spikelets. For each isolate, two rachis branches were inserted in each conical flask or vial for temperature or RH studies, respectively.

To study the effect of temperature on secondary conidia production, spikelets containing honeydew were incubated in the dark for 18 h at 10, 15, 20, 25, 30 and 35 °C in dew chambers at 100 % RH. The effect of RH on secondary conidia production was determined in specially built single plant chambers which offer precise control of RH (Butler *et al.* 1995). The chambers were made of glass jars and moist air was continuously blown in. Depending on the RH requirement, a micrologger (model CR21X; Campbell Scientific, Norah Logan, UT) measured RH in each chamber every 10 s and adjusted it to the desired level by pumping dry air into the chamber (Butler *et al.* 1995). Vials containing spikelets with honeydew were incubated in these chambers in the dark for 18 h at 80, 85, 90, 95, and 100 % RH. The single plant chambers were placed in a growth chamber maintained at 25 °C.

After incubation at different temperature and RH regimes, four infected spikelets with honeydew from each replication were carefully excised from the rachis and collected in 25 ml beakers containing 10 ml sterile water. Spikelets were agitated in water with the aid of a magnetic stirrer to dissolve the honeydew. Macroconidia (1000 per treatment) were observed

under a microscope to determine the proportion of macroconidia that produced secondary conidia. Macroconidia that produced germ tubes with a pointed distal end and were devoid of cytoplasm were considered to have germinated to produce secondary conidia (Bandyopadhyay et al. 1990). Experiments were conducted in a randomized block design with three replications, and repeated three times for a total of nine observations per isolate at each temperature and RH level.

#### Assessment of ergot disease spread in the growth chamber

The relative efficiency of aerial spread of sorghum ergot was compared under controlled conditions in growth chambers using two isolates (NI2 and NAP7) belonging to separate groups. Plants of male sterile sorghum line 296A were grown in 25 cm-diameter plastic pots (two plants per pot) in the greenhouse. Panicles were covered with pollination bags immediately after emergence through boot leaves to avoid contamination from external inoculum. One week before the start of the experiment, six plants (sown 7–10 d earlier than others) were inoculated with either NI2 or NAP7 using the methods described above. Two days after the appearance of honeydew, one pot with each isolate was incubated overnight in separate dew chambers to allow the production of secondary conidia. These infected plants acted as a source of inoculum and were separately placed at the centre of two growth chambers. Twenty-two test plants (the number that could be accommodated in a single growth chamber) at the 50 % flowering stage were placed around each pot containing the inoculum source, and the pollination bags covering the panicles were removed. The growth chambers were maintained at  $25 \pm 1^\circ\text{C}$  and 97–100 % RH with a 12 h photoperiod to encourage secondary conidia production on the inoculum source plants and to allow infection on the test plants. The proportion of infected plants (disease incidence), number of infected spikelets, and total number of spikelets on each plant were recorded 5 d after symptoms appeared. The percent of spikelets infected on each plant was averaged to determine disease severity. The experiment was repeated three times.

#### Statistical analysis

Data were analysed by analysis of variance (ANOVA) using the GENSTAT statistical package (Lane & Payne 1999) and the SAS statistical package (SAS Institute 1999). Analysis of covariance was used in SAS PROC GLM to assess the linear and quadratic effects of temperature and RH on production of secondary conidia. Estimates of linear combinations of parameters ('estimate' statements) were made using PROC GLM to compare Group I and II means and slopes.

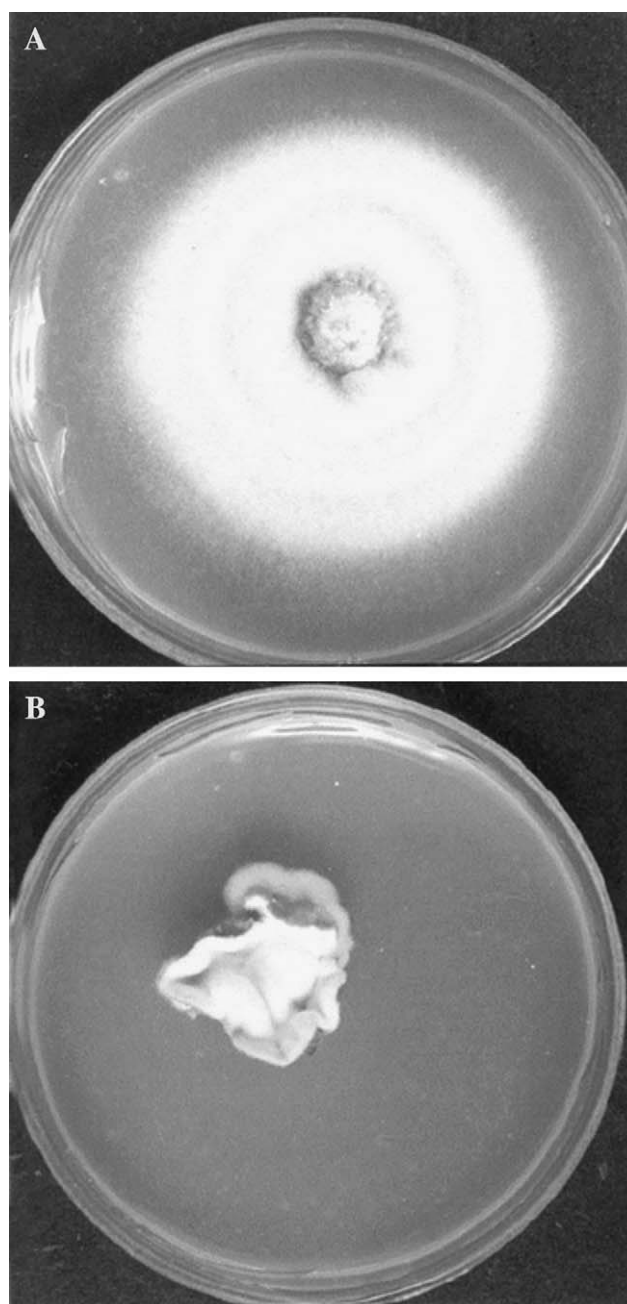
## Results

#### Colony characteristics

Isolates differed in the rate of colony growth, maximum radial growth, colony type, puckering nature (number of sulci) and sporulation on T2 agar medium (Table 1). Growth was cottony,

velvety and submerged with diffused margins in five isolates (NAP4, NAP5, NAP7, NAP13, and MH74; Fig 2A). The remaining isolates had compact, fleshy and raised colonies with distinct margins (Fig 2B). Cultures of all isolates were white. The isolates differed with respect to the sulcate (puckering) nature of the colonies. Of the 89 isolates studied, five isolates (NAP4, NAP5, NAP7, NAP13, and MH74) showed no puckering whereas 47 isolates showed a low degree of puckering, 27 isolates showed a medium degree of puckering, and ten isolates showed a high degree of puckering (Table 1).

Different sorghum ergot isolates produced pigmentation on T2 agar medium ranging from dusky red to light red with a hue and value/chroma reading of 2.5 YR 3/3 to 2.5 YR 6/6



**Fig 2** – Pure cultures of isolate NAP7 (Group II) on T2 agar medium (A); and isolate NI2 (Group I) on T2 agar medium (B).

in Munsell's colour chart (Table 1). Sporulation was observed in cultures of only five isolates (NAP4, NAP5, NAP7, NAP13, and MH74) after 20–25 d of incubation on T2 medium whereas none of the other isolates sporulated on the medium even after 30 d at 25 °C (Table 1). Sporulation was observed at the centre of the colony as minute droplets of honeydew secretions containing both macroconidia and microconidia which were infective on sorghum male sterile line 296A. The secretions were pale brown in colour and were thick in consistency. The maximum radial growth (colony diameter) of colonies of the various isolates ranged from 1.12–3.52 cm (Table 1).

### Grouping of isolates

Isolates were grouped on the basis of growth rate, colony type and sporulation on T2 agar medium. Two distinct groups were observed: Group I consisted of 84 isolates having a slower growth rate (0.03–0.09 mm d<sup>-1</sup>), compact and raised colonies, and Group II contained five isolates (NAP4, NAP5, NAP7, NAP13, and MH74) having a comparatively higher growth rate (0.11–0.12 mm d<sup>-1</sup>), cottony or velvety, submerged colonies with diffused margins and sporulation on T2 agar medium. Eight isolates belonging to Group I and representing geographic diversity within India were selected for further studies: NI2, NI5, NI12, GUJ6, MH71, SK-20-24, AP17, and TN13 (Table 1). Similarly, two isolates (NAP7 and MH74) were chosen as representatives of Group II for comparative studies.

### Sphacelia, conidia and sclerotia

The isolates belonging to Groups I and II varied with respect to morphology of sphacelia, conidia and sclerotia. The sphacelia of Group I isolates measured 2.92–3.60 × 1.90–2.26 mm and were conical to spherical, whereas the sphacelia of Group II isolates measured 4.38–4.72 × 2.94–3.06 mm and were oblong to cylindrical in shape (Table 2, Fig 3A). Group II sphacelia length and breadth measurements were significantly larger ( $P < 0.0001$ ) than those of Group I isolates when compared using 'estimate' statements in Proc GLM in SAS. Both groups had white sphacelia (Table 2).

Macroconidia belonging to the two distinct groups of isolates varied in size and shape. The macroconidia of isolates belonging to Group I were oblong to ellipsoidal with mean length/breadth measurements of 12.9–15.4 × 7.5–8.3 µm (range 10.3–18.0 × 6.4–9.0 µm), whereas, the macroconidia of Group II isolates were narrower, cylindrical, and somewhat smaller with mean length/breadth of 13.1–13.4 × 5.9–6.3 µm (range 9.0–18.0 µm × 5.1–7.7 µm) (Table 3). Length and breadth of macroconidia of Group II isolates were significantly smaller ( $P < 0.0001$ ) than those of Group I isolates when compared with 'estimate' statements in SAS.

Conidia of all isolates were hyaline and showed a slight constriction at the centre with two vacuoles, one at each end. Even though some variation was observed among Group I isolates in microconidia diameter (Table 3), Groups I and II did not differ significantly from one another ( $Pr > t = 0.1739$ ) when group means were compared using 'estimate' statements in SAS. This observation supports the hypothesis that the size and shape of microconidia remains highly consistent throughout the genus *Claviceps* (Pažoutová et al. 2004).

**Table 2 – Morphological characteristics of sphacelia of representative sorghum ergot isolates from Groups I and II**

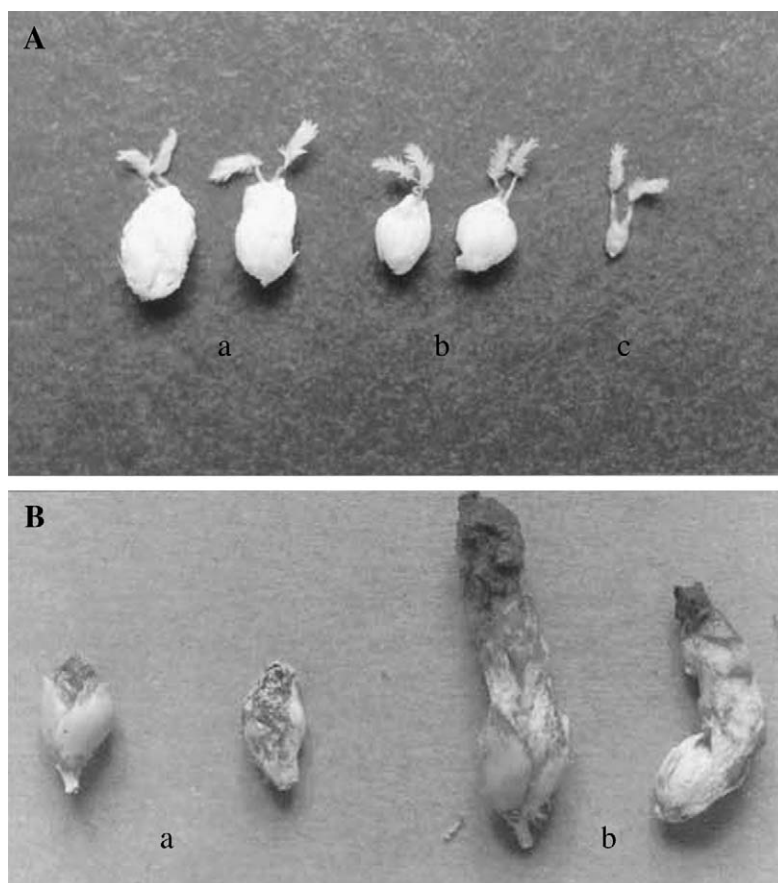
Isolate	Length <sup>a,b</sup> (mm)	Breadth <sup>a,b</sup> (mm)	Shape	Colour
Group I				
NI2	2.92 g	1.96 e	Elliptical to conical	White
NI5	3.02 fg	1.94 e	Elliptical	White
NI12	3.14 ef	2.00 de	Elliptical	White
GUJ6	3.38 d	2.26 c	Spherical to elliptical	White
MH71	3.24 de	1.94 e	Conical	White
SK-20-24	3.60 c	2.08 d	Elliptical to spherical	White
AP17	3.16 ef	1.90 e	Elliptical	White
TN13	3.16 ef	1.92 e	Elliptical	White
Group II				
NAP7	4.72 a	3.06 a	Oblong to elliptical	White
MH74	4.38 b	2.94 b	Oblong to elliptical	White
Least significant difference value (P = 0.05)	0.17	0.12		
Overall means of Groups I and II for length and breadth varied significantly from one another, $P < 0.0001$ .				
<sup>a</sup> Mean of 25 sphacelia.				
<sup>b</sup> Means within a column followed by a common letter(s) are not significantly different at the 5 % level.				

None of the isolates produced sclerotia in at 35 °C and 40 % RH in the controlled environment. Sclerotia collected in the field showed variation in size and shape, although Group I isolates always were observed to have smaller sclerotia compared with Group II isolates (Fig 3B). Sclerotia of Group I isolates with sphacelial cap were conical to spherical with mean length/breadth dimensions of 4.10–4.42 × 1.40–1.82 mm. Sclerotia of Group II isolates with sphacelial cap were cylindrical to conical, with mean length/breadth dimensions of 8.16–9.60 × 1.82–2.04 mm. Length and breadth of sclerotia with sphacelial cap of Group II isolates were significantly greater ( $P < 0.0001$ ) than those of Group I isolates when compared using 'estimate' statements in SAS. Some variation in sclerotial morphological was observed within Groups (Table 4).

Significant differences ( $P = 0.05$ ) were also observed among isolates in the size of sclerotia minus the sphacelial cap. Sclerotia of Group I isolates were 2.20–2.30 × 1.20–1.30 mm and conical to spherical, whereas those of Group II were 3.70–3.90 × 2.00–2.30 mm, oblong to cylindrical (Table 4). The colour of the sclerotia of Group II isolates minus the sphacelial cap was reddish brown with a hue and value/chroma reading of 5YR 5/4 in Munsell's colour chart. Group I sclerotia were reddish brown with a hue and value/chroma of 5YR 4/4, which is darker compared with Group II.

### Production of secondary conidia

The effects of a range of temperatures (10–35 °C) and relative humidity (RH) levels (80–100 %) on secondary conidia



**Fig 3 – Mature sphacelia (A) of two distinct groups: a, isolate NAP7 (Group II); b, isolate NI2 (Group I); c, uninfected ovary. Sclerotia (B) with sphacelial cap: a, isolate SK-20-24 (Group I); b, isolate NAP7 (Group II).**

production was assessed by counting the number of germinated macroconidia (Fig 4). Over a wide range of temperatures and RH, Group II isolates produced significantly fewer secondary conidia compared with Group I isolates (Fig 4). The optimum temperature for secondary conidia production was 25 °C for all isolates. Isolate NI2 (Group I) was the most prolific secondary conidia producer (a mean of 128.8 secondary conidia were produced out of 1000 macroconidia observed at the optimum temperature of 25 °C) and NAP7 (Group II) the least prolific (a mean of 4.4 secondary conidia were produced at 25 °C).

Secondary conidia production varied significantly at different temperatures and among pathogen isolates (Fig 4A). At 20 °C, secondary conidia production by all isolates followed the same trend observed at 25 °C. At 15 °C, secondary conidia production was reduced for almost all isolates except AP17, which produced its maximum number of secondary conidia (mean = 27.56) at this temperature. Few secondary conidia were produced at the lowest (10 °C) and highest temperatures (35 °C) examined. Isolates varied significantly in their linear and quadratic slope estimates (Table 5), indicating that the intensity of the response to temperature varied among isolates. Most of the variation was observed within Group I isolates as such low overall macroconidia germination was observed for Group II isolates. Using 'estimate' statements in SAS,

significant differences ( $P < 0.0001$ ) were observed between Groups 1 and II in overall means, and in linear and quadratic slopes.

Secondary conidia production also varied with RH levels (Fig 4B). As for temperature, all isolates belonging to Group I produced more secondary conidia than Group II (11) isolates. Isolate NI2 (Group I) produced the maximum number of secondary conidia (111.3 at 25 °C and 100 % RH), followed by NI12, while the isolates belonging to Group II produced low numbers of secondary conidia even at the most favourable conditions (25 °C and 100 % RH). Maximum secondary conidia production was observed at high RH in all the isolates tested. With a decrease in RH, secondary conidia production showed a decreasing trend with the lowest amount observed at 80 % RH. The rate of increase in secondary conidia production was comparatively low between 80 and 95 % RH and high between 95 and 100 % RH. Isolates varied significantly in their linear and quadratic slope estimates (Table 5). As for temperature, 'estimate' statements were used in SAS to compare overall means and linear and quadratic slopes of Group I versus Group II isolates and the groups significantly differed ( $P < 0.0001$ ) in each case. As observed for the temperature response, most of the observed variation was within the Group I isolates, as low overall macroconidia germination was observed for the Group II isolates.

**Table 3 – Morphological characteristics of macroconidia and microconidia of representative sorghum ergot isolates from Group I and Group II**

[illegible]

### Disease spread in the growth chamber

The mean disease incidence was 71.2 % for plants infected with isolate NI2 (Group I) and 15.1 % for plants infected with isolate NAP7 (Group II), with an least significant difference (LSD) value ( $P = 0.05$ ) of 18.8 %. Disease severity was 5.36 for NI2 and 0.42 for NAP7 with an LSD value of 2.9. Substantially more plants adjacent to the focus of infection were infected compared with those that were more distant from the focus of infection.

## Discussion

This study has provided the opportunity for a comparison of two distinct groups of *Claviceps* isolates causing sorghum ergot in India. From our observations of the morphology of Group I and Group II isolates from India, and in light of prior descriptions that compared morphology of the two species originating from different continents (Frederickson et al. 1991;

**Table 4 – Morphological characteristics of sclerotia (with and without sphacelial cap) of sorghum ergot isolates in Group I and Group II**

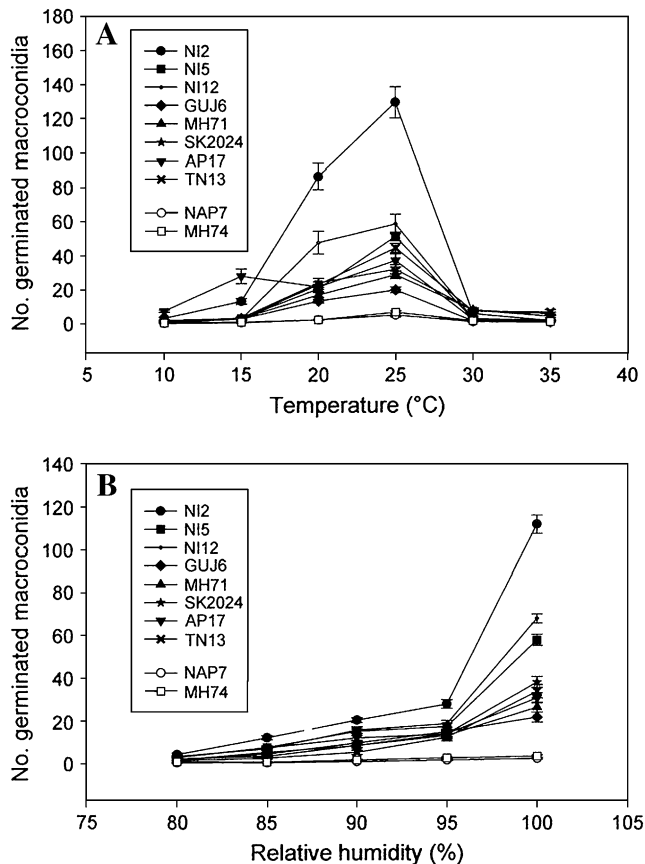
Isolate	Sclerotia with sphacelial cap				Sclerotia without sphacelial cap			
	Length <sup>a,b</sup> (mm)	Breadth <sup>a,b</sup> (mm)	Shape	Colour	Length <sup>a,b</sup> (mm)	Breadth <sup>a,b</sup> (mm)	Shape	Colour
Group I								
NAP12	4.42 d	1.82 b	Conical to spherical	LYB <sup>c</sup>	2.20 b	1.30 c	Conical to spherical	RB <sup>d</sup>
AK1	4.40 d	1.40 c	Conical to spherical	LYB	2.20 b	1.20 c	Conical to spherical	RB
SK-20-24	4.10 e	1.94 ab	Spherical to conical	LYB	2.30 b	1.20 c	Conical to spherical	RB
Group II								
NAP4	9.40 a	1.84 b	Cylindrical to conical	LYB	3.80 a	2.00 ab	Oblong to cylindrical	RB
NAP5	8.16 c	1.84 b	Cylindrical to conical	LYB	3.80 a	2.10 a	Oblong to cylindrical	RB
NAP7	9.12 b	2.04 a	Cylindrical to conical	LYB	3.70 a	2.30 a	Oblong to cylindrical	RB
NAP13	9.60 a	1.82 b	Cylindrical to conical	LYB	3.90 a	2.20 a	Oblong to cylindrical	RB
Least significant difference <sub>(P = 0.05)</sub>	0.21	0.147			0.266	0.223		

<sup>a</sup> Mean of 25 sclerotia.

<sup>b</sup> Means within a column followed by same letter(s) are not significantly different at the 5 % level. Overall means of Groups I and II for length and breadth of sclerotia with sphacelial cap varied significantly from one another,  $P < 0.0001$  based on estimates of linear combinations of parameters ('estimate' statements) in PROC GLM within SAS (SAS Institute 1999).

<sup>c</sup> LYB, Light yellowish brown. Pigmentation was recorded using Munsell's colour chart (Munsell Products 1973).

<sup>d</sup> RB, Reddish brown.



**Fig 4 – Effects of temperature (A) and relative humidity (B) on secondary conidia production measured by assessing germination of macroconidia of representative isolates from Group I and Group II.** The upper eight isolates on each graph (designated by solid symbols) represent Group I isolates while the lower two (designated by open symbols) represent Group II isolates. Data are means of nine observations (three replications in each of three experiments with 1000 macroconidia assessed per replication) per isolate–temperature or isolate–relative humidity combination. Standard error bars are shown on the graphs.

Kulkarni et al. 1976) we conclude that Group I isolates represent *Claviceps africana* and Group II isolates represent *C. sorghi*. Based on our 2000 survey of sorghum ergot in India, we found five isolates to represent *C. sorghi* and the remaining 84 to be *C. africana*. The *C. sorghi* isolates occurred from the states of Andhra Pradesh and Maharashtra. Pažoutová and Bogo (2001) also collected a *C. sorghi* isolate from Gulbarga in the state of Karnataka. It thus appears that *C. sorghi* is still present within India, but at a low frequency compared with *C. africana*. It has been suggested that *C. sorghi* was gradually replaced by *C. africana* in India due to the propensity for *C. africana* to spread more quickly due to its the large-scale production of secondary conidia (Bandyopadhyay et al. 2002; Pažoutová et al. 2000). Results of our work appear to support this hypothesis. However, there appear to be some localized areas in India, such as drought-prone northern Karnataka, where *C. sorghi* remains more prevalent than *C. africana* (Johnson & Rajasab 2003).

Notable differences in cultural characteristics and morphology were observed among the Group I (*C. africana*) and Group II (*C. sorghi*) isolates we collected. The growth of *C. sorghi* isolates was cottony and velvety with aerial hyphae, and cultures were non-sulcate. Similar observations were reported by Ramakrishnan (1948) and Nagarajan and Saraswathi (1975) in describing *C. sorghi*. We found the *C. sorghi* isolates to sporulate on Kirchoff's medium, and then infect sorghum male sterile line 296A. Similar results were obtained by Chinnadurai (1972). But contrary to their reports of profuse sporulation of *C. sorghi* on Kirchoff's medium, we found sporulation to be sparse on T2 agar medium. This may be attributed to the differences in the medium used, or may suggest genotypic variation among isolates. The cultural characters we observed in the Group I (*C. africana*) isolates we collected were quite different from those of *C. sorghi* and in agreement with those described by Bogo and Mantle (1999). *C. africana* isolates were compact, fleshy, white, sulcate (with a low to high degree of puckering) and failed to sporulate on T2 medium. No puckering was observed in *C. sorghi* isolates.

The rate of growth of *C. africana* isolates was low compared with that of *C. sorghi*. Sphacelia of the *C. sorghi* isolates were substantially greater in length and breadth than those of *C. africana*. The sphacelia of *C. sorghi* isolates were white, bilaterally grooved, and more cylindrical than those of *C. africana*. This is in agreement with observations made by Sangitrao and Bade (1979) and Frederickson et al. (1991). Sphacelia of *C. africana* were white and oval to spherical in shape, in agreement with the work of Frederickson et al. (1991). *C. africana* sphacelial lengths ranged from 2.92–3.60 mm, which contrasts with observations (5–8 mm) of Frederickson et al. (1991). This difference may be due to variation in ovary size of the sorghum genotypes used in the two studies.

Our study supports the observations made by Kulkarni et al. (1976) and Frederickson et al. (1991) on macroconidial morphology in *C. sorghi* and *C. africana*, respectively. Macroconidia of *C. sorghi* and *C. africana* isolates differed distinctly. While the conidia of the former were more cylindrical, measuring  $9\text{--}18 \times 5.1\text{--}7.7\ \mu\text{m}$ , the latter were oblong measuring of  $9\text{--}18 \times 5.1\text{--}9\ \mu\text{m}$ . The shape and size of the microconidia recorded in this study agree with the dimensions given by Kulkarni et al. (1976), Bandyopadhyay et al. (1990) and Frederickson et al. (1991). In our study no significant variation in shape and size of microconidia was observed in different sorghum ergot isolates. Similar observations were made by Sangitrao (1982) and Frederickson et al. (1991).

None of the isolates we studied produced sclerotia in infected sorghum male sterile line 296 A following artificial inoculation, even under very favourable environmental conditions for sclerotial production in growth chambers. This may be attributable to an unfavourable host genotype on which no sclerotial production occurred. However, sclerotia formed under natural conditions in seven ergot isolates collected during the survey. The sclerotia obtained from farmers' fields during the survey were formed on sorghum cultivars CSH 9, MSH 51 and JK 22. These genotypes therefore appear to favour sclerotial production upon infection by the pathogen under optimum environmental condition. Sangitrao (1982) attributed the variation in the size of sclerotia to variation in host genotypes that favour sclerotial production. In our study,

**Table 5 – Intercepts, linear and quadratic slopes for evaluating effects of temperature and relative humidity (RH) on secondary conidia production by representative Group I and Group II *Claviceps* isolates collected in India<sup>a</sup>**

Isolate	Temperature (10–35 °C)			Relative humidity (80–100%)		
	Intercept	Linear slope	Quadratic slope	Intercept	Linear slope	Quadratic slope
Group I						
NI2	–227.08	27.60	–0.61	3106.73	–73.34	0.43
NI5	–77.47	9.15	–0.20	1328.91	–31.68	0.19
NI12	–112.16	13.47	–0.30	1738.33	–41.20	0.24
GUJ6	–31.08	3.92	–0.09	104.55	–3.12	0.02
MH71	–44.30	5.39	–0.12	383.98	–9.49	0.06
SK 20–24	–54.79	6.63	–0.14	723.00	–17.39	0.11
AP17	–40.64	6.59	–0.16	617.22	–14.90	0.09
TN13	–68.27	8.07	–0.17	915.49	–21.72	0.13
Group II						
NAP7	–6.66	0.77	–0.02	33.69	–0.83	0.01
MH74	–8.30	1.01	–0.02	31.20	–0.79	0.01
Least significant difference, $P = 0.01^b$		7.089	0.152		10.25	0.11

<sup>a</sup> Analysis of covariance was used to assess the linear and quadratic effects of temperature and RH on production of secondary conidia using SAS Proc GLM (SAS Institute 1999).

<sup>b</sup> Least significant difference value for statistically comparing linear or quadratic slopes among isolates.

sclerotia that formed on the same sorghum cultivar (CSH 9) infected by either *C. sorghi* or *C. africana* varied distinctly in their length, suggesting the possible role of genetic variation in the production of sclerotia by *C. sorghi* and *C. africana*. Sclerotia of *C. sorghi* were larger than those of *C. africana* (Table 4), and the size of the sclerotia was further influenced by the amount and length of sphacelial tissue present on the surface of the sclerotia. However, sclerotial colour both with and without the sphacelial cap was identical in the two species.

*C. africana* isolates produced secondary conidia more profusely than *C. sorghi* isolates in our study. However, the amount of secondary conidia production within *C. africana* isolates varied not only between isolates but also at different temperature and RH regimes. In *in vitro* studies in Australia, the temperature optimum for secondary conidia production by *C. africana* was reported as 20 °C and no secondary conidia were produced at  $\geq 30$  °C (Bhuiyan *et al.* 2002). Our *in vivo* studies showed 25 °C and 100 % RH as optimal conditions for secondary conidia production. Secondary conidia also were produced at 30 and 35 °C, but at a much lower frequency. Thus, *C. africana* isolates not only had the ability to produce large numbers of secondary conidia under optimal conditions, but also could produce a sufficient number of secondary conidia over a range of environmental conditions (15–35 °C and 85–100 % RH). Variation in secondary conidia production among isolates within the groups across temperature regimes may be attributable to the climatic adaptations of the isolates to their respective locality of origin. We observed that the isolates originating from northern India (Uttar Pradesh and Rajasthan states) produced greater numbers of secondary conidia at 25 °C compared with isolates of southern Indian origin. This may be attributed to the similar environmental conditions (slightly warmer and more humid) that prevail in northern India, to which the isolates may have become adapted. In addition, at 35 °C isolates MH71, SK-20-24 and TN13 produced larger numbers of secondary conidia compared

with other isolates from northern India (NI2, NI5 and NI12). This may be due to the existence of higher temperatures ( $>30$  °C) in the region of origin of such isolates.

Though sorghum ergot may spread through ascospores and macroconidia, spread by secondary conidia is of prime concern in the epidemiology of the disease. Indeed, secondary conidia have been implicated in the rapid spread of *C. africana* (Frederickson *et al.* 1993; Bandyopadhyay *et al.* 1998). In many cases, failure of germination by sclerotia is reported (Bandyopadhyay *et al.* 1996). In the absence of ascospores, and rain-splash to disseminate the macroconidia, the spread of the disease is effectively carried out through airborne secondary conidia. Differences we observed in secondary conidia production between *C. africana* and *C. sorghi* isolates corresponded to the existence of higher levels of airborne spread of *C. africana* versus *C. sorghi* in the growth chamber experiments. Profuse production of secondary conidia by *C. africana* and negligible production by *C. sorghi* is in agreement with earlier findings (Frederickson *et al.* 1989; Bandyopadhyay *et al.* 1996).

The cultural, morphological and epidemiological evidence we collected in determining which of our isolates represent *C. africana* versus *C. sorghi* was supported by evidence from AFLP and DNA sequencing studies which also clearly distinguished *C. africana* from *C. sorghi* (Tooley *et al.* 2006). This study also suggests that a previous report (Bandyopadhyay *et al.* 1990) on the biology of the ergot pathogen in India dealt with *C. africana* and not *C. sorghi* as described in the report.

Of the 89 isolates collected and studied in the present investigation only five isolates (NAP 4, NAP 5, NAP 7, NAP 13, and MH 74) were *C. sorghi* and 84 isolates were *C. africana*, indicating the vast predominance of *C. africana* in major sorghum growing areas of India during the period in which these collections were made. *C. sorghi*, being found in such low frequency in India, having low propensity to spread through secondary conidia, and not reported to occur elsewhere in the world, currently appears to represent little

threat to other major sorghum producing regions of the world.

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## REFERENCES

- Ajrekar SL, 1926. Observations on a disease of Jowar (*Sorghum vulgare*) caused by *Sphacelia* (conidial stage *Claviceps*). *Journal of the Indian Botanical Society* 5: 55–61.
- All India Coordinated Sorghum Improvement Project (AICSIP), 1969–1970. *Progress report of the all India coordinated sorghum improvement project*. 1969–70. Indian Council of Agricultural Research and Cooperative Agencies, New Delhi, pp. 138–146.
- Bandyopadhyay R, Mughogho LK, Manohar SK, Satyanarayana MV, 1990. Stroma development and honeydew formation, and conidial production in *Claviceps sorghi*. *Phytopathology* 80: 812–818.
- Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN, 1996. Ergot—a global threat to sorghum. *International Sorghum and Millet Newsletter* 37: 1–32.
- Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN, Ryley MJ, 1998. Ergot: a new disease threat to sorghum in the Americas and Australia. *Plant Disease* 82: 356–367.
- Bandyopadhyay R, Muthusubramanian V, Tooley PW, Chakraborty S, Pažoutová S, Navi SS, 2002. Distribution and diversity of the sorghum sugary disease pathogens in India. In: Leslie JF (ed), *Sorghum and Millet Diseases 2000*. Iowa State University Press, Ames, IA, pp. 75–78.
- Bhuiyan SA, Galea VJ, Ryley MJ, Tay D, Lisle AT, 2002. Factors influencing the germination of macroconidia and secondary conidia of *Claviceps africana*. *Australian Journal of Agricultural Research* 53: 1087–1094.
- Bogo A, Mantle PG, 1999. *Claviceps africana* discovered in India. *Plant Disease* 83: 79.
- Butler DR, Reddy RK, Wadia KR, 1995. Single-plant chambers to control humidity for pathological studies. *Plant Pathology* 44: 1–9.
- Chinnadurai G, 1972. Effect of certain trace elements on the growth and sporulation of *Sphacelia sorghi*. *Indian Phytopathology* 25: 599–600.
- Frederickson DE, Mantle PG, De Milliano WAJ, 1989. Secondary conidiation of *Sphacelia sorghi* on sorghum, a novel factor in the epidemiology of ergot disease. *Mycological Research* 93: 497–502.
- Frederickson DE, Mantle PG, de Milliano WAJ, 1991. *Claviceps africana* sp. nov., the distinctive ergot pathogen of sorghum in Africa. *Mycological Research* 95: 1101–1107.
- Frederickson DE, Mantle PG, de Milliano WAJ, 1993. Windborne spread of ergot disease (*Claviceps africana*) in sorghum A-lines in Zimbabwe. *Plant Pathology* 42: 368–377.
- Frederickson D, Odvody G, Isakeit T, 1999. *Sorghum Ergot: Distinguishing Sphacelia and Sclerotia of Claviceps africana in Seed* (Publication No. L-5315.). Agricultural Communications, Texas A&M University System, College Station, TX.
- Lane PW, Payne RW, 1999. *Genstat for Windows: an Introductory Course*, 4th edn. Numerical Algorithms Group, Oxford.
- Johnson N, Rajasab AH, 2003. Prevalence of *Claviceps sorghi* in Karnataka, India. *International Sorghum and Millets Newsletter* 44: 93–95.
- Komolomb B, Chakraborty S, Ryley M, Yates D, 2002. Identity and genetic diversity of the sorghum ergot pathogen in Australia. *Australian Journal of Agriculture Research* 53: 621–628.
- Kulkarni GS, 1942. Ergot in India. *Current Science* 11: 246.
- Kulkarni BGP, Seshadri VS, Hegde RK, 1976. The perfect stage of *Sphacelia sorghi* McRae. *Mysore Journal of Agricultural Science* 10: 286–289.
- Munsell Products, 1973. *Munsell Soil Color Charts Macbeth Color & Photometry Division*. Kollmorgen Corporation, Baltimore, MD.
- Nagarajan K, Saraswathi V, 1975. Production of honeydew line secretions in the culture of *Sphacelia sorghi*. *Indian Phytopathology* 28: 110.
- Nath P, Padwick GW, 1941. Ergot in India. *Current Science* 10: 488–489.
- Navi SS, Bandyopadhyay R, Nageswara Rao TG, Tooley PW, 2002a. An outbreak of sorghum ergot in parts of Andhra Pradesh, India. *International Sorghum and Millets Newsletter* 43: 68–70.
- Navi SS, Bandyopadhyay R, Tonapi VA, Nageshwar Rao TG, Tooley PW, Reddy RK, Indira S, Pande S, 2002b. Prevalence of ergot of sorghum in India. *International Sorghum and Millets Newsletter* 43: 70–71.
- Patil PY, Raut NK, Deshpande PM, 1968. A note on the occurrence of ergot; sugary disease on the hybrid Jowar in Maharashtra (Parbhani). *College of Agriculture, Parbhani Magazine* 4: 93–94.
- Pažoutová S, Bandyopadhyay R, Frederickson DE, Mantle PG, Frederiksen RA, 2000. Relations among sorghum ergot isolates from the Americas, Africa, India and Australia. *Plant Disease* 84: 437–442.
- Pažoutová S, Bogo A, 2001. Rediscovery of *Claviceps sorghi* (Ascomycotina: Clavicipitaceae) in India. *Mycopathologia* 153: 99–101.
- Pažoutová S, Kolarik M, Kolinska R, 2004. Pleomorphic conidiation in *Claviceps*. *Mycological Research* 108: 126–135.
- Ramakrishnan TS, 1947. The natural occurrence of ergot in south India. *Proceedings Indian Academy of Sciences* 26: 136–141.
- Ramakrishnan TS, 1948. Ergot sclerotia on *Sorghum vulgare* Pers. *Current Science* 17: 218.
- Sangitrao CS, 1982. *Studies on ergot disease of sorghum in Vidarbha*. PhD thesis, Punjabrao Krishi Vidyapeeth, Akola, India.
- Sangitrao CS, Bade GH, 1979. Meteorological factors associated with honeydew development and sclerotial stage in sorghum ergot. *Sorghum Newsletter* 22: 107–108.
- SAS Institute, 1999. *SAS/STAT. Version 8 manual*. SAS Institute, Cary, NC.
- Sundaram NV, 1969. Current plant pathological problems in new agricultural strategy with special reference to sorghum and millets. *Indian Phytopathological Society Bulletin* 4: 62–72.
- Thomas KM, Ramakrishnan TS, Shrinivasan KV, 1945. Natural occurrence of ergot in south India. *Proceedings of the Indian Academy of Sciences, B* 21: 93–100.
- Tooley PW, O'Neill NR, Goley ED, Carras MM, 2000. Assessment of diversity in *Claviceps africana* and other *Claviceps* species by RAM and AFLP analyses. *Phytopathology* 90: 1126–1130.
- Tooley PW, Goley ED, Carras MM, O'Neill NR, 2002. AFLP comparisons among *Claviceps africana* isolates from the United States, Mexico, Africa, Australia, India, and Japan. *Plant Disease* 86: 1247–1252.
- Tooley PW, Bandyopadhyay R, Carras MM, Pažoutová S, 2006. Analysis of *Claviceps africana* and *C. sorghi* from India using AFLPs, EF-1 $\alpha$  gene intron 4 and  $\beta$ -tubulin gene intron 3. *Mycological Research* 110: 441–451.
- Tsukiboshi T, Shimanuki T, Uematsu T, 1999. *Claviceps sorghicola* sp. nov., a destructive ergot pathogen of sorghum in Japan. *Mycological Research* 103: 1403–1408.