

## VARIATION IN MORPHOLOGY AND PATHOGENICITY IN *CLAVICEPS FUSIFORMIS*, THE CAUSAL AGENT OF PEARL MILLET ERGOT

BY S. S. CHAHAL\*, V. P. RAO AND R. P. THAKUR

*Pearl Millet Improvement Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P. O., A.P. 502 324, India*

Isolates of the pearl millet ergot pathogen *Claviceps fusiformis* were collected from eight locations in India and studied for morphological and pathogenic variations. Sclerotia were examined for shape, size and cavities, and residual macro- and microconidia for their size and viability. Sclerotia from Aurangabad and ICRISAT Centre were the largest ( $5 \times 2.5$  mm) and heaviest (1.4 g/100 sclerotia), and from Mysore the smallest ( $3 \times 2$  mm) and lightest (0.4 g/100 sclerotia). The average number of cavities (furrows) was minimum in the Mysore collection (4/sclerotium) and maximum in the Kovilpatti collection (15/sclerotium). Large variations occurred in size and ratios between the number of macro- and microconidia residual on sclerotia from different locations. Time to initiation of germination of macroconidia from sclerotia in sterile distilled water at 25 °C varied from 24 h in Aurangabad and Jobner collections to 50 h in the Kovilpatti collection. The macroconidia of the Pune collection did not germinate. The isolates differed in growth characteristics on Kirchoff's agar at 25°. In a pathogenicity test using 10-day-old culture inoculum in six pearl millet genotypes (3 resistant and 3 susceptible), some isolates were more virulent than others.

Ergot of pearl millet (*Pennisetum americanum* (L.) Leeke) caused by *Claviceps fusiformis* Loveless is an important disease accounting for a considerable loss in grain production every year. In India, the disease is severe, and all commercial hybrids and varieties are highly susceptible (Anon., 1975-83). In addition to reducing yield, ergot adversely affects the quality of the produce by contaminating the grain with toxic alkaloid-containing sclerotia of the fungus (Bhat, Roy & Tulpule, 1976; Kannaiyan, Vidhyasekaran & Kandaswamy, 1971; Krishnamachari & Bhat, 1976). Of the various control measures suggested (Singh & Singh 1969; Kannaiyan, Vidhyasekaran & Kandaswamy, 1973; Sundaram, 1975; Brar, Chand & Thakur 1976; Thakur, Williams & Rao, 1982) use of host-plant resistance is the most desirable approach. Efforts to develop ergot resistant lines following pedigree selection in the progenies of crosses involving ergot low-susceptible lines (Thakur, Rao & Williams, 1982) and to breed ergot resistant cultivars through recurrent selection (Gill, Chahal & Phul, 1980; Chahal, Gill, Phul & Singh, 1981) have been successful. The success however, depends on their performance under a wide range of environments

and against variable populations of the pathogen. In multilocation testing, five lines developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) near Hyderabad India, showed less than 1% ergot severity at ICRISAT Centre and Jamnagar, but developed 8-61% ergot severity at Aurangabad, Pune, Kano, and Samaru (ICRISAT, 1982). Such differential reactions of the genotypes could be attributed to several factors including variations in environmental conditions and in the pathogen population at different locations. At the ICRISAT Centre, Hyderabad, we studied morphological and pathogenic variation among nine isolates of *C. fusiformis* from eight Indian locations as reported in this paper.

### MATERIALS AND METHODS

#### *Collection and preservation of isolates*

Sclerotia of *C. fusiformis* were collected from eight locations in India; Aurangabad (ABD), Kovilpatti (KPT), Jobner (JBN), Coimbatore (CBR), Ludhiana (LDH), Pune (PUN), ICRISAT Centre (ICR) and Mysore (MYS). The locations include the extreme north - LDH (30° 56' N) and the extreme south - KPT (9° 12' N), representing the major pearl millet growing areas in India. Ergot appears in severe form at these locations every year.

\* Visiting Scientist at ICRISAT; Present address: Plant Pathologist, Dept. of Plant Breeding, Punjab Agricultural University, Ludhiana, Punjab, India.

Collections were made during October from the rainy season crops of 1981 or 1982 from two commercial pearl millet hybrids NHB-3 or BJ-104. Sclerotia from each location were refrigerated at 5–6° in sealed polythene bags for 3–12 months.

### Morphology

Size (length and width) and 100 sclerotium weight from each collection were measured. The number of cavities (furrows) present in sclerotia was observed in transverse sections from the base, middle and upper portions of three sclerotia taken at random from each collection. To determine the ratios between macro- and microconidia, aqueous suspensions of equal concentration prepared from the sclerotia were examined microscopically, and ten counts were taken for each collection. Macro- and microconidia (50 each) from each collection were examined for shape and size. To examine conidial germination, a drop of the above conidial suspension in a cavity slide was incubated in a moist chamber at 25°. Three cavity slides were maintained for each collection.

Axenic culture of each collection was obtained from the sclerotia surface sterilized with 0.1% mercuric chloride for 2 min, washed with sterile distilled water, cut into small pieces, and plated on Kirchoff's agar (Kirchoff, 1929) in Petri dishes. Plates were then incubated at 25° with 12 h alternate light and dark for 10 days.

### Pathology

Using a standard inoculation method (Thakur & Williams, 1980) two ergot-resistant (ICMPE-71 and ICMPE-37) and two susceptible (BJ-104 and BK-560) genotypes in field, and one resistant (ER-43) and one susceptible (PHB-10) genotype in the screenhouse, were inoculated with each of the nine isolates. Approximately equal volumes (5–6 ml) of an aqueous conidial suspension ( $10^6$  conidia/ml), obtained from 10-day-old cultures containing mostly macroconidia (> 90%), except CBR-B and PUN isolates which produced only microconidia, were used to inoculate each inflorescence. Water-sprayed inflorescences were maintained as checks. Two replications of five inflorescences each were used for each isolate. To maintain high humidity, sprinkler irrigation was provided twice daily for 30 min periods in the field and thrice daily for 10 min periods in the screenhouse.

Inflorescences were harvested 20 days after inoculation, and scored for ergot severity using the standard ergot severity assessment key (Thakur & Williams, 1980).

## RESULTS

### Morphology

*Sclerotia.* Sclerotia were round at the base, broad in the middle and tapered towards the tip in all but the KPT collection in which sclerotia had a tapered base and were rarely curved and irregularly broad at the tip. Sclerotia in the MYS collection were round at the base giving them a pyriform appearance (Fig. 1). Based on measurements of length and width and 100-sclerotial weight, the collections were grouped in four categories: (1) ABD and ICR; (2) LDH and CBR; (3) JBN, PUN and KPT and (4) MYS. Sclerotia from category 1 were the largest in size (5 × 2.5 mm) and heaviest (1.4 g/100 sclerotia) and those from category 4 the smallest (3 × 2 mm) and lightest (0.4 g/100 sclerotia) (Table 1).

There was less variation in width than in length of sclerotia. In MYS, 68% of the sclerotia were 3–4 mm long. Twenty percent of the sclerotia in CBR, 30% in LDH, 33% in ABD, 50% in PUN, 52% in JBN and 60% in KPT were 4–5 mm in length. In the ICR isolate, 40% of the sclerotia were 5–6 mm long. Frequency of sclerotia with 2–3 mm width was highest across the collections. Sclerotia from MYS were shorter than those from the others (Fig. 2). The average number of cavities per sclerotium across collections was 6–7 (ranging from 4 in MYS to 15 in KPT), being least in the lower part, more in the middle, and most in the upper part.

*Conidia from sclerotia.* Macroconidia were hyaline, fusiform to falcate with tapered ends, larger in size in LDH, ABD, PUN and ICR than in JBN, MYS, KPT and CBR collections, greater in width in ABD than in other collections. Microconidia were hyaline, rounded to elongated, more elongated in KPT than in other collections. Most collections had fewer macroconidia than microconidia, the lowest proportion being 1:157 in the MYS. In contrast to sclerotium residual conidia, the freshly exuded honeydew contains mostly macroconidia. In the KPT collection, however, macroconidia outnumbered microconidia 25 to 1 (Table 2).

### Germination of conidia from sclerotia

Time to initiation of germination of macroconidia from different collections varied from 24 h (ABD, JBN) to 50 h (KPT) in sterile distilled water at 25°. Macroconidia germinated by producing 1–3 germ-tubes either from the sides or apices (Fig. 3a). Macroconidia in the ICR collection generally produced more than three (even up to eight) germ-tubes (Fig. 3b). However, in the KPT

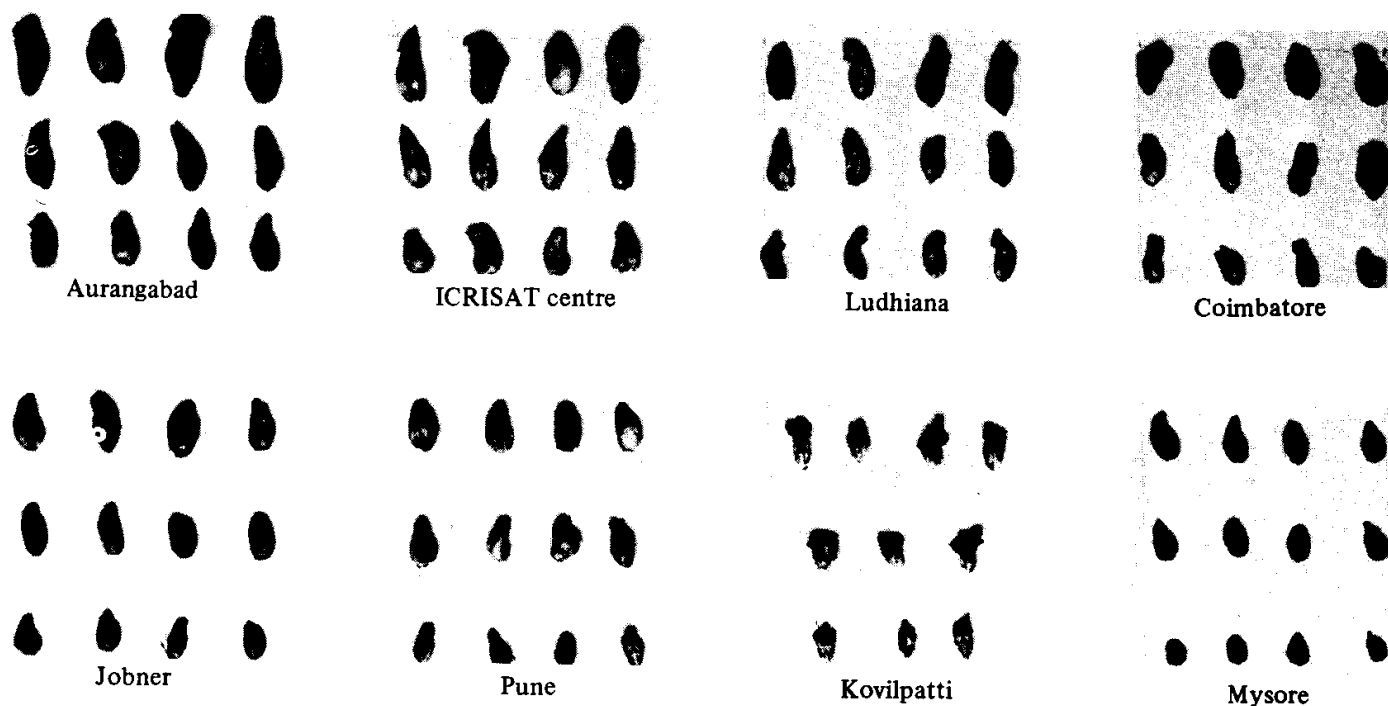


Fig. 1. Morphological variation in *Claviceps fusiformis* sclerotia from eight locations in India.

Table 1. Variation in size (length  $\times$  breadth) and weight of pearl millet ergot sclerotia from different locations (isolates)

Location (isolate)	Size (mm)*		100-sclerotial weight (g)
	Mean	Range	
Aurangabad (ABD)	5.1 $\times$ 2.3	3.9 $\times$ 2.4	1.4
ICRISAT centre (ICR)	5.1 $\times$ 2.5	3.8 $\times$ 1.4	1.5
Ludhiana (LDH)	4.7 $\times$ 2.1	3.8 $\times$ 2.4	1.0
Coimbatore (CBR)	4.5 $\times$ 2.1	3.6 $\times$ 1.5	1.2
Jobner (JBN)	4.1 $\times$ 2.1	3.6 $\times$ 2.3	0.9
Pune (PUN)	3.9 $\times$ 2.5	3.6 $\times$ 2.4	0.8
Kovilpatti (KPT)	3.8 $\times$ 2.3	3.5 $\times$ 2.4	0.7
Mysore (MYS)	3.1 $\times$ 1.9	2.6 $\times$ 1.4	0.4
S.E.M.	0.3 $\times$ 0.2	—	0.2

\* Based on measurements of 100 sclerotia.

collection apical germ-tubes were rare and only one or two straight, long, side germ-tubes were normally produced (Fig. 3c). Sometimes a rounded secondary conidium was produced at the tip. Macroconidia from the PUN collection did not germinate.

Budding or division was commonly observed in microconidia and was initiated after 19 h in PUN to 28 h in ABD collections at 25°. Budding or division of one or more secondary conidia from one or both ends was also common (Fig. 4a-c). Microconidia in JBN and MYS collections divided and further subdivided to form a characteristic pattern (Fig. 4d). Chains of small tertiary conidia were formed in PUN collection (Fig. 4e).

#### Colony characteristics

On Kirchoff's agar at 25° mycelial growth (from the slices of sclerotia) became visible in ABD, CBR, ICR, JBN, LDH and PUN isolates after 38-40 h, and in KPT and MYS isolates after 60 h. Ten-day-old colonies of ABD, ICR, JBN, LDH, MYS and KPT isolates were irregularly-circular to circular with white mycelia. The colonies were flat to slightly raised, compact, smooth, entire, translucent and creamish on the reverse side. Colonies of the PUN isolate were circular, lobed, dirty-white to pinkish-grey, with slimy ooze on the surface, dirty-pink to greenish-grey towards the margin and with concentric rings on the reverse. In cultures of

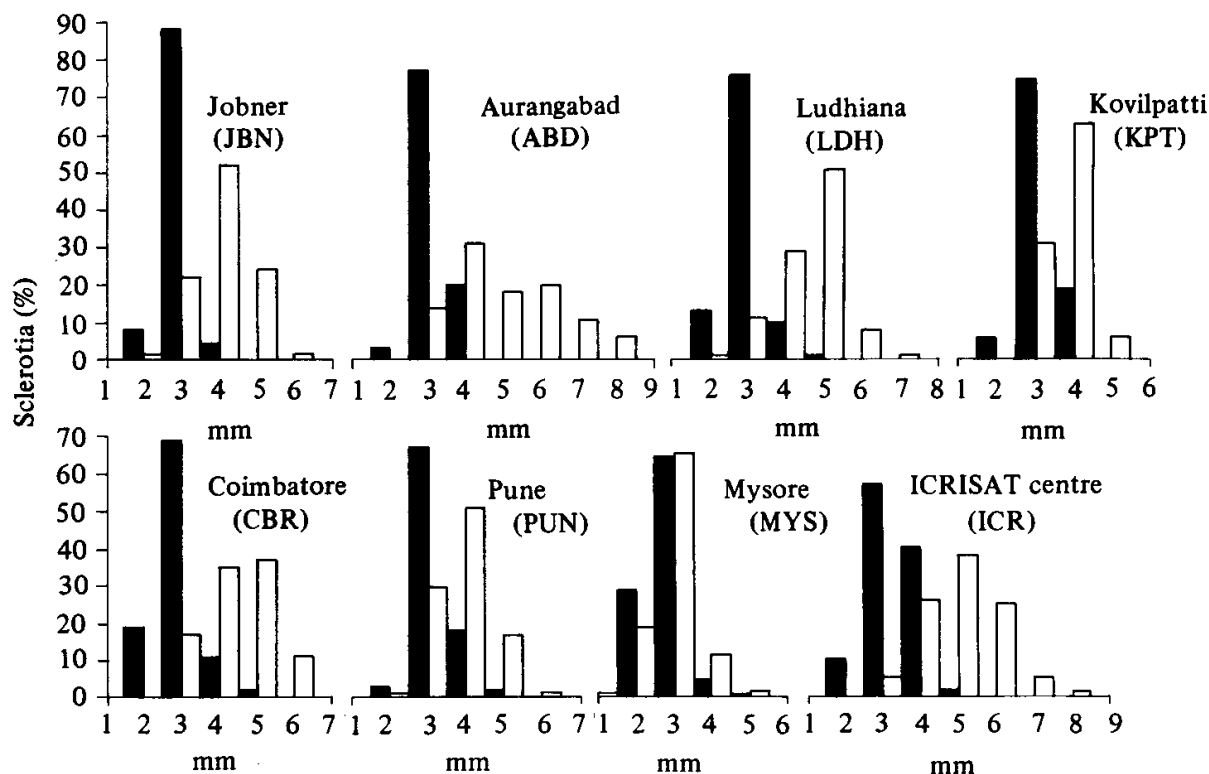


Fig. 2. Frequency distribution of pearl millet ergot sclerotial size from eight locations in India, □, length; ■, breadth.

Table 2. Variation in size (length  $\times$  breadth) and ratios between number of macro- and microconidia from ergot sclerotia from different locations

Location (isolate)	Macroconidia* ( $\mu\text{m}$ )		Microconidia* ( $\mu\text{m}$ )		Macro:Micro
	Mean	Range	Mean	Range	
Ludhiana (LDH)	26.4 $\times$ 4.6	20-34 $\times$ 4-6	6.2 $\times$ 4.4	5-10 $\times$ 4-6	1:34
Aurangabad (ABD)	24.5 $\times$ 6.2	17-33 $\times$ 4-7	6.6 $\times$ 5.4	5-9 $\times$ 4-7	1:15
Pune (PUN)	24.3 $\times$ 4.4	20-30 $\times$ 4-6	6.1 $\times$ 4.1	4-8 $\times$ 4-5	1:97
ICRISAT Centre (ICR)	23.8 $\times$ 4.3	16-28 $\times$ 4-6	6.0 $\times$ 4.4	4-8 $\times$ 4-6	1:34
Jobner (JBN)	22.8 $\times$ 4.1	19-32 $\times$ 3-5	5.8 $\times$ 4.3	4-7 $\times$ 4-5	1:29
Mysore (MYS)	22.1 $\times$ 4.1	18-27 $\times$ 4-6	6.0 $\times$ 4.2	4-8 $\times$ 4-6	1:157
Kovilpatti (KPT)	20.9 $\times$ 4.1	16-28 $\times$ 4-6	7.5 $\times$ 4.0	5-10 $\times$ 3-5	25:1
Coimbatore (CBR)	20.5 $\times$ 5.4	18-26 $\times$ 4-6	6.4 $\times$ 5.5	6-8 $\times$ 5-6	1:2
S.E.M.	0.4 $\times$ 0.07	—	0.1 $\times$ 0.08	—	—

\* Based on measurements of 50 conidia.

all the isolates, macroconidia were produced within 6-7 days and microconidia within 8-10 days except in the PUN isolate where only microconidia were produced.

Of 37 CBR colonies, 29 were similar to the other isolates and the remaining 8 to the PUN isolate. The former type was designated CBR-A and the latter CBR-B. Measurements of macro- and microconidia of the isolates from the culture were comparable to those from sclerotia except PUN and CBR-B which produced only microconidia in axenic culture.

#### Pathogenicity

The KPT isolate showed maximum virulence across host-genotypes both in field and screenhouse tests. On susceptible genotypes ergot severities (mean and range) produced by KPT, JBN, LDH and ABD isolates were not significantly different, although PHB-10 scored generally less ergot than BJ-104 and BK-560 (Table 3). Similarly, isolates CBR-A, ICR and MYS did not differ significantly from each other on susceptible genotypes. On resistant genotypes, although isolate KPT differed

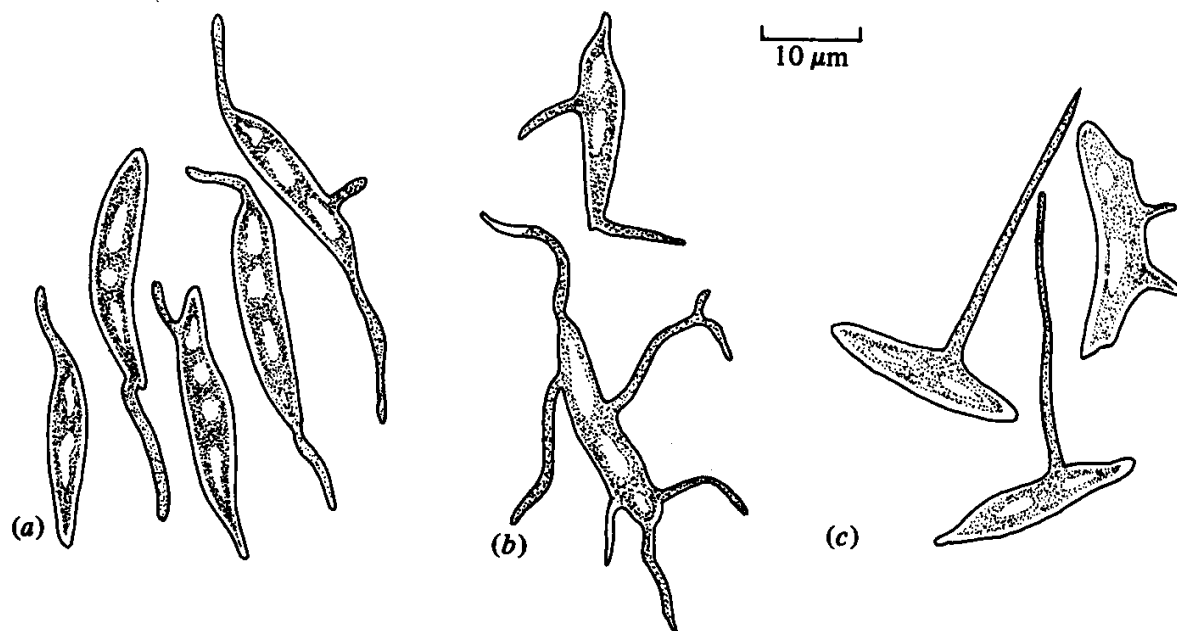


Fig. 3. Mode of germination of macroconidia in isolates of *Claviceps fusiformis*: (a) 1-3 germ-tubes common in all the isolates, (b) more than 3 germ-tubes (ICR isolate) (c) straight, long, side germ-tube (KPT isolate).

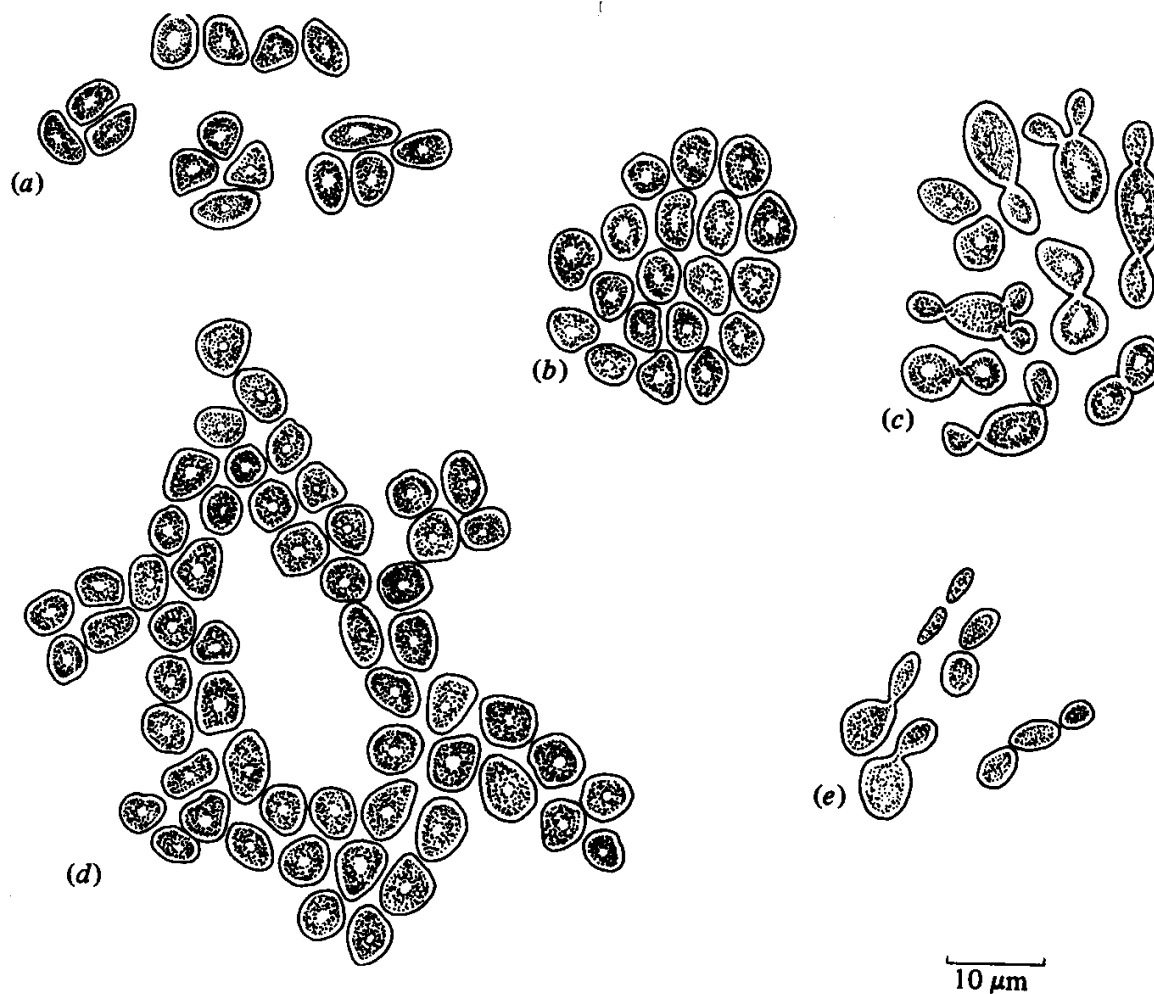


Fig. 4. Budding and division in microconidia in isolates of *Claviceps fusiformis* (a-c), (d) characteristic pattern for JBN and MYS isolates, (e) tertiary conidia of the PUN isolate.

Table 3. Mean ergot severity percent\* in six pearl millet genotypes following inoculation with seven isolates of *Claviceps fusiformis*

Isolate	Susceptible genotype			Resistant genotype		
	BJ-104†	BK-560†	PHB-10‡	ICMPE-71†	ICMPE-37†	ER-43‡
	Mean	Mean	Mean	Mean	Mean	Mean
	Range	Range	Range	Range	Range	Range
KPT	90 (72)§	82 (65)	54 (46)	18 (23)	9 (17)	20 (25)
JBN	75 (60)	77 (61)	66 (54)	1 (5)	1 (3)	4 (11)
LDH	82 (65)	73 (59)	51 (46)	4 (11)	2 (10)	6 (13)
ABD	68 (56)	59 (50)	54 (46)	<1 (2)	1 (6)	7 (16)
CBR-A	29 (32)	27 (30)	33 (33)	<1 (2)	5 (11)	2 (7)
ICR	28 (32)	31 (33)	17 (23)	1 (5)	<1 (2)	2 (9)
MYS	28 (32)	19 (26)	15 (23)	2 (7)	1 (5)	3 (10)
C.D. (5%)	18 (12.6)	24.4 (16)	25 (14)	13.3 (14)	7 (10.5)	13.2 (10.4)

\* Mean of 10 inflorescences in each genotype.

† Field experiment.

‡ Screenhouse experiment

§ Arcsin transformed value.

significantly from all others on ICMPE-71, there were no significant differences between KPT, LDH and CBR-A on ICMPE-37 and between KPT and ABD on ER-43. Two isolates, CBR-B and PUN did not induce any disease symptoms.

#### DISCUSSION

Large morphological variations were found in sclerotia of different collections. These could be attributed to environmental factors and the host genotype from which the sclerotia were collected. In our studies, sclerotia were collected from either of two host genotypes highly susceptible to ergot at each location. Temperature and relative humidity, two factors which greatly influence ergot development, varied considerably from location to location. To avoid confounding effects of host  $\times$  environment interactions, it would be useful to study the variations of the isolates on a single host genotype in one environment.

Although morphological variations were found in sclerotia from different collections, conidial measurements fell within the range reported for *C. fusiformis* (Loveless, 1967). Sclerotia contained both macro- and microconidia but the ratios between the number of macro- and microconidia varied among the isolates. The isolates with more macroconidia appeared to be more virulent than those with fewer macroconidia, indicating a positive correlation between the number of macroconidia and the degree of virulence of the isolate (Table 2). In our studies, although sclerotia were preserved at 5–6°, the effects of storage time and temperatures on the viability of macro- and microconidia were not investigated.

In the PUN isolate, macroconidia from sclerotia did not germinate (probably these were not viable), but microconidia produced numerous secondary and tertiary conidia as in the other isolates. Production of microconidia in chains from the tips of the germ tubes of macroconidia (Thakur, Rao & Williams, 1984) and tertiary conidia has also been reported by Siddiqui & Khan (1973), but their role in ergot epidemiology has not been established.

On the basis of pathogenicity tests the seven isolates may be divided into highly virulent (KPT, JBN, LDH and ABD) and weakly virulent (CBR-A, ICR and MYS) groups. Two of the isolates, CBR-B and PUN, did not induce disease symptoms, probably because they contained only microconidia. This raises a further question about the role of microconidia in ergot epidemiology.

From the CBR isolate on Kirchoff's medium two morphological types were distinguished. The types also differed in pathogenicity, one being avirulent and the other virulent. This indicated a possible

inherent variation in the pathogen which needs further investigation. In the case of *C. purpurea* different virulent types from one isolate on male-sterile spring wheat have been reported (Darlington, Mathre & Johnston, 1977) and Mantle, Shaw & Doling (1977) also identified on male sterile wheat highly and weakly infective groups among *C. purpurea* isolates from graminaceous hosts.

Although variations in virulence of the isolates were observed on the basis of the severity of ergot infection on resistant and susceptible genotypes, there is a need for further investigation to confirm this. Our studies provide basic information on the extent of variation in *C. fusiformis* in India, and raise several questions for future investigations.

The authors gratefully acknowledge Dr S. B. King, Principal Millet Pathologist, ICRISAT, for his comments on the manuscript.

Submitted as Journal Article no. 430 by International Crops Research Institute for the Semi-Arid Tropics, (ICRISAT).

#### REFERENCES

- ANON. (1975–83). Progress Reports of the All India Coordinated Millet Improvement Project. *Indian Council of Agricultural Research and Cooperating Agencies*.
- BHAT, R. V., ROY, D. N. & TULPUL, P. G. (1976). The nature of alkaloids of ergoty pearl millet or bajra and its comparison with alkaloids of ergoty rye and ergoty wheat. *Toxicology and Applied Pharmacology* **36**, 11–17.
- BRAR, G. S., CHAND, J. N. & THAKUR, D. P. (1976). Fungicidal control of ergot of bajra. *Haryana Agricultural University Journal of Research* **6**, 1–5.
- CHAHAL, S. S., GILL, K. S., PHUL, P. S. & SINGH, N. B. (1981). Effectiveness of recurrent selection for generating ergot resistance in pearl millet. *SABRAO Journal* **13**, 184–186.
- DARLINGTON, L. C., MATHRE, D. E. & JOHNSTON, R. H. (1977). Variation in pathogenicity between isolates of *Claviceps purpurea*. *Canadian Journal of Plant Sciences* **57**, 729–733.
- GILL, K. S., CHAHAL, S. S. & PHUL, P. S. (1980). Strategy to develop ergot resistance in pearl millet. In *Trends in Genetical Research in Pennisetums* (ed. V. P. Gupta & J. L. Minocha), pp. 159–161. Punjab Agricultural University, Ludhiana.
- ICRISAT (1982). *Annual Report 1981*. International Crops Research Institute for the Semi-Arid Tropics, Patancheru P.O. A.P. 502 324, India pp. 71.
- KANNAIYAN, J., VIDHYASEKARAN, P. & KANDASWAMY, T. K. (1971). Mammalian toxicity of ergot of bajra. *Current Science* **40**, 557–558.
- KANNAIYAN, J., VIDHYASEKARAN, P. & KANDASWAMY, T. K. (1973). Effect of fertilizers application on the incidence of ergot disease of bajra (*Pennisetum typhoides* B and S). *Indian Phytopathology* **26**, 355–357.

- KIRCHOFF, H. (1929). Contributions to the biology and physiology of the ergot fungus. Abstract in *Review of Applied Mycology* 8, 560-562 (Transl. from German).
- KRISHNAMACHARI, K. A. V. R & BHAT, R. V. (1976). Poisoning by ergoty bajra (pearl millet) in man. *Indian Journal of Medical Research* 64, 1624-1628.
- LOVELESS, A. R. (1967). *Claviceps fusiformis* sp. nov., the causal agent of an agalactica of sows. *Transactions of the British Mycological Society* 50, 15-18.
- MANTLE, P. G., SHAW, S., & DOLING, D. A. (1977). Role of weed grasses in the etiology of ergot disease in wheat. *Annals of Applied Biology* 86, 339-351.
- SIDDIQUI, M. R. & KHAN, I. D. (1973). Dynamics of inoculum and environment in relation to ergot incidence on *Pennisetum typhoides* (Burm.) Stapf. and Hubbard. *Transactions of the Mycological Society Japan* 14, 280-288.
- SINGH, R., & SINGH, S. N. (1969). A note on effects of different dates of sowing hybrid bajra-1 on grain yield and incidence of ergot (*Claviceps microcephala* (Wallr.) Tul.). *Madras Agriculture Journal* 56, 140.
- SUNDARAM, N. V. (1975). Ergot of bajra. In *Advances in Mycology and Plant Pathology*, pp. 155-160. Published by Professor R. N. Tandon's birthday celebration committee, New Delhi.
- THAKUR, R. P. & WILLIAMS, R. J. (1980). Pollination effects on pearl millet ergot. *Phytopathology* 70, 80-84.
- THAKUR, R. P., WILLIAMS, R. J. & RAO, V. P. (1982). Development of resistance to ergot in Pearl millet. *Phytopathology* 72, 406-408.
- THAKUR, R. P., RAO, V. P., & WILLIAMS, R. J. (1984). The morphology and disease cycle of ergot caused by *Claviceps fusiformis* in pearl millet. *Phytopathology* 74, 201-205.

(Received for publication 18 October 1983)