Expression of Ergot Resistance in Pearl Millet Under Artificially Induced Epidemic Conditions

R. P. Thakur, S. B. King, and V. P. Rao

Senior plant pathologist, principal plant pathologist, and research associate, respectively, Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.
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ABSTRACT

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Resistance to ergot (*Claviceps fusiformis*) in pearl millet, previously identified by using an inoculation technique for field screening, was tested under artificially induced epidemics in the field and greenhouse. The epidemic conditions were created by repeated spray inoculation of protogynous panicles with an aqueous honeydew conidial suspension (1×10^6 conidia/ml) under conditions of high relative humidity (>90%) or panicle wetness (>80%) and moderate temperature (20–30 C). There were no significant differences in ergot reactions under artificially induced epidemic conditions and field screening for resistant (≤10% mean ergot severity) or susceptible (≥30% mean ergot severity) cultivars. However, cultivars that were moderately resistant (10–30% ergot severity) in field screening showed very low ergot (<2% mean severity) that were comparable to the highly resistant cultivars, under epidemic conditions both in field and greenhouse. The results suggest that an ergot severity threshold level of 20–30% in field screening should provide adequate levels of functional field resistance under natural ergot epidemic conditions. Pearl millet cultivars with moderate levels of ergot resistance and having desirable agronomic traits may be suitable for cultivation in areas where ergot is of economic importance.

Additional keyword: Pennisetum glaucum.

Identification of resistance to a plant disease under artificially induced epidemics does not necessarily ensure that the resistance will remain effective under natural epidemic conditions. Field screening of pearl millet (*Pennisetum glaucum* (L.) R. Br.) for resistance to ergot (*Claviceps fusiformis* Loveless) at ICRISAT Center is done by inoculating, at the protogny stage, individual panicles that are protected from cross-pollination by bagging before and after inoculation (9). This screening technique has been used in the breeding and identification of a number of ergot-resistant cultivars (6,9). Resistance in many of these cultivars has been stable in multilocational testing in India and West Africa over several years (7).

Although we believe that essential components for successful screening for resistance to ergot in pearl millet include elimination of pollen interference and timely inoculation within a suitable environment, we also recognize the necessity to determine whether resistance identified in this manner holds under natural conditions of ergot epidemics. However, because ergot epidemics are infrequent, localized, and unpredictable, it is necessary to resort to methods that, as much as possible, simulate the conditions believed to be characteristic of ergot epidemics. Generally high humidity (RH>90%), frequent rain showers, moderate temperatures (20–30 C), and overcast skies during flowering favor ergot development in pearl millet (1–5). From our experience at ICRISAT Center, we believe that wet weather and moderate temperature are the most important environmental factors for ergot development.

Our objectives in this study were to test the resistance of some ergot-resistant pearl millet cultivars under induced disease epidemic conditions in the field and greenhouse and to determine their functional levels of field resistance.

MATERIALS AND METHODS

Terminology. Three inoculation methods were involved in this study.

Field screening is defined as inoculation of individual panicles at full protogyny with a honeydew conidial suspension (1×10^6 conidia/ml), using a hand-held pressure sprayer, and protection of panicles from extraneous pollen by covering them with parchment paper bags, before and after inoculation. This is the field-based screening technique developed and used at ICRISAT Center to identify ergot resistance (9). High RH is created by providing overhead sprinkler irrigations twice a day for 30 min each on rain-free days, starting from the first date of inoculation until 1 wk after the last date of inoculation.

Field epidemic is defined as mass field inoculation of open panicles at protogyny with a honeydew conidial suspension (1×10^6 conidia/ml), using a motorized knapsack power sprayer. Panicles are not bagged, and high RH is created by two applications of sprinkler irrigation on rain-free days.

Greenhouse epidemic is defined as inoculation of open panicles with a honeydew conidial suspension (1×10^6 conidia/ml), using a hand-held sprayer in a greenhouse. Panicles are not bagged, and temperatures are maintained at 20–30 C, and misters, controlled by a data logger attached to leaf wetness sensors or RH sensors, are used to maintain free water on panicles and a high RH within the greenhouse bay.

Field experiments. Nine pearl millet cultivars, one susceptible (≥30% mean severity) to ergot, WC-C75, three moderately resistant (10–30% mean severity), ICMPES 8, ICMPES 9, and ICMPES 32; and five resistant (≤10% mean severity), ICMPES 5, ICMPES 23, ICMPES 28, ICMPES 29, and ICMPES 34, were tested for ergot reaction in three field experiments at ICRISAT Center. Because the cultivars varied in number of days to flowering, sowing dates were adjusted so that flowering in all the cultivars occurred at about the same time. These cultivars were tested both under field screening and field epidemic conditions.

Field screening. During the 1984, 1987, and 1988 rainy seasons, each cultivar was grown in a 2-row plot of 4-m length and replicated twice in a randomized block design. Rows were spaced at 75 cm and plants at 15 cm within rows. Normal agronomic practices were followed. In each row, 10 panicles (main panicle/plant) were inoculated. Panicles were scored for ergot severity,

using an ergot severity rating scale (8), 20 days after inoculation, and mean ergot severity was calculated for each cultivar.

Field epidemic. *Evaluation of ergot resistance.* During the 1984 rainy season, each cultivar was grown in 4-row plots of 4-m length, while in the 1987 and 1988 rainy seasons each was grown in 8-row plots of 8-m length. A randomized block design was used with three replications in 1984 and 1988, and four in 1987. The other conditions were similar to those described above for field screening. The first inoculation was made when about 25% of panicles had reached the protogyny stage, and inoculation was continued for the following 10 days, until all panicles had completed protogyny. Only the main shoot panicles were evaluated for ergot. Inoculations were made during the evening between 1600 and 1700 hours to avoid midday heat. Panicles were scored for ergot severity in the central two rows in 1984 and the central four rows in 1987 and 1988, 20 days after inoculation.

A hygrothermograph (British Rototherm Co. Ltd., England) was stationed 1 m above ground level to monitor daily temperature and RH during the course of the experiments.

Evaluation of agronomic traits. In 1987 and 1988, each cultivar was evaluated for time to 50% flowering, number of tillers per plant, plant height, panicle length, 1000-grain mass, and grain yield. In the two central rows of each plot, 10 plants (main shoot), 1 after every 10 in a row, were selected for measuring agronomic traits, except 1000-grain mass, and grain yield. At crop maturity, panicles were harvested from the central 6 m of four central rows of each plot, sun-dried, threshed, cleaned, and grain weight taken for each plot. Adjusted grain yield was calculated for a uniform plant stand of 160 plants in each 4 row × 6 m (3 × 6 m) plot area. Grain samples were drawn from each plot and 1000-grain mass was determined.

Greenhouse experiments. In experiment 1, pot-grown plants of four pearl millet cultivars, one susceptible to ergot (BJ 104), one moderately resistant (ICMPES 8), and two resistant (ICMPES 5, ICMPES 34) were transferred to a greenhouse at the boot-leaf stage. Each cultivar was grown in 10 plastic pots (20-cm-diameter) with two to three plants per pot, and the pots were arranged in a completely randomized design. The main shoot panicles were spray inoculated at the protogyny stage, and the tillers were removed before flowering to avoid extraneous pollen. The inoculated panicles were marked to indicate the times of inoculation and of anthesis for each panicle, so that the time interval between inoculation and anthesis on any given panicle was known. Inoculation was continued for several days until all test panicles had reached protogyny.

Environmental conditions favorable for ergot epidemics were maintained by operating overhead misters, controlled by a data logger connected to leaf wetness sensors adjusted to provide >80% wetness, and adjusting evaporative coolers to maintain temperatures at 20–30 C. Temperature, RH, and leaf wetness were recorded by a CR-21 data logger (Campbell Scientific Co., Logan, UT) and psychrometers. Plants were transferred to another greenhouse 6 days after the last inoculation. The first panicles inoculated were thus exposed to the wet conditions for 10 days and the last ones for 6 days (the latent period for ergot being 4–6 days). Panicles were scored for ergot severity and seed set 20 days after inoculation.

In experiment 2, pot-grown plants of seven pearl millet cultivars, three susceptible to ergot (BK 560, 841A, WC-C75), two moderately resistant (ICMPES 8, ICMPES 9), and two resistant (ICMPES 29, ICMPES 34), were spray-inoculated and exposed to the same set of conditions as in experiment 1. In this experiment, however, each cultivar was tested separately to reduce the extent of cross-pollination, and consequently reduce the interference with ergot infection. Each cultivar was grown in 20 pots with two to three plants per pot. The other details were the same as in experiment 1.

RESULTS

Field experiments. *Comparison of ergot resistance between field screening and field epidemic conditions.* The susceptible cultivar, WC-C75, had a mean ergot severity of 72% under field screening and 24% under the induced field epidemic condition. The three moderately resistant cultivars had mean ergot severities of 12–23% under field screening, but showed very little ergot (1–2% mean severity) under the epidemic condition. The four resistant cultivars had mean ergot severities of 2–5% under field screening and were all either ergot-free or had <1% mean ergot severity under the epidemic condition (Table 1).

Evaluation for agronomic traits. Data on agronomic traits, such as time to 50% flowering, tillers per plant, plant height, panicle length, 1000-grain mass, and grain yield of eight resistant or moderately resistant cultivars and WC-C75, a susceptible, commercial cultivar, are presented in Table 2. All the resistant and moderately resistant cultivars flowered significantly later than WC-C75. The

TABLE 1. Evaluation of pearl millet cultivars susceptible (S), moderately resistant (MR), and resistant (R) to ergot under field screening and under artificially induced epidemics in field experiments at ICRISAT Center

Cultivar	Known ergot reaction[c]	Ergot severity (%)							
		Field screening[a]				Artificial epidemic[b]			
		1984	1987	1988	Mean	1984[d]	1987[e]	1988[f]	Mean
WC-C75	S	73	83	59	72 ± 6.9[g]	45	11	27	24 ± 10.4[g]
ICMPES 8	MR	19	21	24	21 ± 1.4	<1	<1	4	2 ± 1.2
ICMPES 9	MR	18	28	24	23 ± 2.9	<1	1	1	1 ± 0.2
ICMPES 32	MR	9	14	13	12 ± 1.5	1	1	<1	1 ± 0.2
ICMPES 5	R	2	2	<1	2 ± 0.5	0	0	0	0
ICMPES 23	R	1	3	1	2 ± 0.6	<1	1	<1	1 ± 0.2
ICMPES 28	R	1	9	1	4 ± 2.6	0	<1	0	<1 ± 0.1
ICMPES 29	R	1	12	1	5 ± 3.6	0	<1	0	<1 ± 0.1
ICMPES 34	R	<1	5	<1	2 ± 1.5	0	1	0	<1 ± 0.2

[a]Mean of 30–40 panicles from two replications in 1984 and 1987 and from three replications in 1988; field screening of individual panicle was done by inoculation at protogyny and the panicles were protected from cross-pollination by covering them with parchment selfing bags before and after inoculation in the ergot nursery.
[b]Artificial ergot epidemic was created by daily mass inoculation of all the cultivars using a knapsack power sprayer for several days starting at 25% flowering until 100% flowering, high RH, or prolonged wetness was maintained by providing overhead sprinkler irrigations twice daily at noon and in the evening, and panicles were not bagged.
[c]Based on several years of multilocational testing (by field screening method) through ICRISAT's International Pearl Millet Ergot Nursery (IPMEN); S = ≥30% mean ergot severity; MR = 10–30% mean ergot severity; R = ≤10% mean ergot severity.
[d]Mean of 100–110 panicles from three replications.
[e]Mean of 460–700 panicles from four replications.
[f]Mean of 502–814 panicles from three replications.
[g]Standard error.

resistant and moderately resistant cultivars were comparable to WC-C75 for tillers per plant, plant height, and panicle length. Although the 1000-grain mass in some of the resistant and moderately resistant cultivars was significantly lower than in WC-C75, the grain yields of seven of the eight cultivars were comparable to WC-C75.

Evaluation of resistance under greenhouse epidemic conditions. *Genotypes tested simultaneously.* The susceptible cultivar, BJ 104, recorded significantly higher ergot severity (15%) and significantly lower seed set (10%) than moderately resistant and resistant cultivars (Table 3). Ergot severity of a moderately resistant cultivar, ICMPES 8, was, however, not significantly different from that of BJ 104. The resistant cultivars that showed 1% ergot severity, however, had only 28–44% seed set.

The time periods between inoculation and occurrence of anthesis on individual panicles were quite variable among and within cultivars. In the susceptible cultivar, BJ 104, the average time period between inoculation and anthesis was 97 hr, compared with 49 hr in the moderately resistant cultivar, ICMPES 8, and 24–38 hr in the two resistant cultivars, ICMPES 5 and ICMPES 34 (Table 3).

Genotypes tested separately. The three susceptible cultivars sustained 41–58% ergot severity compared with 14 and 19% in the two moderately resistant cultivars, and 1 and 6% in the two resistant cultivars (Table 4). Accordingly, there was less seed set in the susceptible cultivars than in the moderately resistant and resistant cultivars; ICMPES 34 had the maximum seedset of 74%.

DISCUSSION

Environmental conditions that are favorable for development of a plant disease epidemic in nature are extremely difficult to create in a growth chamber, greenhouse, or in the field. Our attempts to create conditions that influence the development of ergot in pearl millet were certainly no exception to this problem. In field and greenhouse experiments, our attempts to create ergot epidemic conditions by repeated spray inoculation, provision of high RH by overhead sprinkler or mist irrigations, and average daily air temperature in the range of 20–32 C were conducive for ergot infection and development (1–3). The two most important environmental factors, RH and temperature, were within the reported optimal range for disease development. Under artificially induced disease epidemics in both the field and greenhouse, susceptible, moderately resistant and resistant cultivars generally behaved the same as they would in field screening, except that the moderately resistant cultivars showed ergot severities similar to those of resistant cultivars under inoculated field conditions. Another exception involved the open-pollinated cultivar WC-C75, which showed large variation in mean ergot severity from 72% under field screening to 24% under field epidemic conditions. This variation in ergot severity under the two conditions is important and significant from an epidemiological point of view. The reduced ergot severity in WC-C75 under field epidemic conditions can be attributed mainly to the cross-pollination-based escape resistance (8) so important in a natural epidemic situation. Cross-pollination, of course, does not occur in field screening since panicles are bagged. The fact that moderately resistant cultivars remained resistant under artificial field epidemic conditions suggests, however, the potential effective value of this level of resistance under natural ergot epidemic conditions.

Field screening of pearl millet cultivars for ergot resistance, by using the inoculation method (9), can be criticized as being too severe and unnatural. However, we argue in favor of the inoculation method of field screening because it gives more reliable and repeatable results that are not confounded by cross-pollination-based escape resistance. We also recognize, however, that this method precludes the possibility of identifying functional levels of field resistance. The results (Table 1) suggest that a level of 20–30% ergot severity under field screening (by inoculation) would probably suffice for functional field resistance. Therefore, cultivars that consistently show this range of ergot severity under field screening in multilocational testing probably would provide adequate levels of field resistance under natural epidemic conditions.

It was expected that under conditions of continuous wetness in the greenhouse, anther dehisence and pollination might be

TABLE 3. Evaluation of pearl millet cultivars susceptible (S), moderately resistant (MR), and resistant (R) to ergot for the time period between inoculation and anthesis, and for ergot reaction and seed set under artificially induced ergot epidemic[a] conditions in a greenhouse.

Cultivar	Known ergot reaction[b]	Time between inoc. and anth. (hr)[c]	Ergot severity (%)[c]	Seed set (%)[c]
BJ 104	S	97 (40-100)[d]	15	10
ICMPES 8	MR	49 (16-90)	12	24
ICMPES 5	R	24 (16-46)	1	28
ICMPES 34	R	38 (16-94)	1	44
SE (m)			±3.1	±4.1

[a]Ergot epidemic was created in greenhouse by maintaining continuous wetness, using overhead misters on inoculated panicles at 30–35/20–25 C day/night temperatures.
[b]Based on multilocational testing (by field screening method) for several years through the ICRISAT's International Pearl Millet Ergot Nursery (IPMEN); S = ≥30% mean ergot severity; MR = 10–30% mean ergot severity; R = ≤10% mean ergot severity.
[c]Mean of 20–40 panicles.
[d]Range of time periods based on 20–30 panicles.

TABLE 2. Mean performance of nine pearl millet cultivars susceptible (S), moderately resistant (MR), and resistant (R) to ergot for agronomic traits under artificially induced ergot epidemic conditions in field during the 1987 and 1988 rainy seasons at ICRISAT Center

Cultivar	Time to 50% flowering (days)	Tillers/ plant (no.)[a]	Plant height (cm)[a]	Panicle length (cm)[a]	1000 grain mass (g)	Grain yield (kg ha^{-1})[b]
WC-C75 (S)[c]	40.0	2.1	195.5	22.5	8.2	1994
ICMPES 8 (MR)	48.5	2.3	182.0	24.5	7.5	1872
ICMPES 9 (MR)	53.5	2.5	189.5	21.5	6.6	1786
ICMPES 32 (MR)	51.0	2.1	168.0	26.0	6.3	1644
ICMPES 5 (R)	47.5	2.4	185.5	27.5	5.0	1602
ICMPES 23 (R)	54.0	2.2	152.0	31.5	6.6	1223
ICMPES 28 (R)	52.0	2.1	187.5	31.5	8.1	2258
ICMPES 29 (R)	53.5	2.2	183.5	29.0	7.0	1686
ICMPES 34 (R)	46.5	1.7	204.0	27.5	6.6	1929
Mean	49.6	2.1	183.0	26.5	6.9	1776.5
SE (m)±	1.0	0.25	5.40	1.15	0.35	189.3
C.V. (%)	5	21	7	10	12	23

[a]Based on 10 plants per plot.
[b]Adjusted yield based on uniform plant stand of 160 plants per plot from a plot size of 18 m^2 in a randomized block design with four replications in 1987 and three replications in 1988.
[c]S = ≥30% mean ergot severity; MR = 10–30% mean ergot severity; R = ≤10% mean ergot severity.

TABLE 4. Ergot severity and seed set in pearl millet cultivars, susceptible (S), moderately resistant (MR), and resistant (R) to ergot under artificially induced epidemic[a] in a greenhouse at ICRISAT Center

Cultivar	Known ergot reaction[b]	Ergot severity (%)[c]	Seed set (%)[c]
BK 560	S	41 ± 3.5[d]	6 ± 2.5[d]
841A	S	58 ± 4.4	0
WC-C75	S	41 ± 5.5	20 ± 6.4
ICMPES 8	MR	14 ± 2.0	36 ± 3.9
ICMPES 9	MR	19 ± 2.4	63 ± 3.6
ICMPES 29	R	1 ± 0.6	38 ± 4.9
ICMPES 34	R	6 ± 1.9	74 ± 3.9

[a]Ergot epidemic was created by continuous wetness provided by operating overhead misters on inoculated panicles at 30–35/20–25 C day/night temperatures.
[b]Based on several years of multilocational testing (by field screening method), in the ICRISAT's International Pearl Millet Ergot Nursery (IPMEN); S = ≥30% mean ergot severity; MR = 10–30% mean ergot severity; R = ≤10% mean ergot severity.
[c]Mean of 20–60 panicles.
[d]Standard error.

reduced considerably, which would result in very high levels of ergot severity. This, obviously, did not happen based on the extent of seed set in each genotype. It seems that even under continuous rain showers the process of anther dehisence and pollination continues, though perhaps to a lesser extent. The degrees of seed set in different cultivars are clear indications of their self compatibility for pollination and fertilization at the individual panicle level. With limited air movement in the greenhouse bay, cross-pollination between plants was likely minimal and, therefore, the extent of seed set on individual panicles was largely due to self-pollination.

The two ergot-resistant cultivars, ICMPES 5 and ICMPES 34, recorded the lowest ergot severity and the highest seed set. The lower ergot severity and higher seed set in resistant and moderately resistant cultivars than in the susceptible cultivar also can be attributed to differences in their floral biology, particularly in relation to the length of time between inoculation and anthesis. In this study, the time periods between inoculation and anthesis were 24–38 hr in the ergot-resistant cultivars, and 97 hr in the susceptible cultivar, supporting earlier findings (10).

All resistant and moderately resistant cultivars tested in this study are sib-bulk populations and, therefore, they are likely similar in the extent of their genetic diversity as most open-pollinated varieties. Seven of the eight ICMPES lines produced grain yields comparable to WC-C75, several also compared favorably for other agronomic traits, such as tiller number, plant height, panicle length, and 1000-grain mass, but they generally flowered and matured later than WC-C75. These and other ICMPES lines available at ICRISAT Center could be evaluated for their agronomic performance and disease resistance in different agroclimatic zones, in view of their possible use as varieties in ergot-endemic areas.

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