#### Resistance

# Development of Resistance to Ergot in Pearl Millet

R. P. Thakur, R. J. Williams, and V. P. Rao

Plant pathologist, principal pathologist, and technical assistant, respectively, Pearl Millet Improvement Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., Andhra Pradesh 502 324, India. ICRISAT Journal Series Article 170. Accepted for publication 18 June 1981.

#### ABSTRACT

Thakur, R. P., Williams, R. J., and Rao, V. P. 1982. Development of resistance to ergot in pearl millet. Phytopathology 72:406-408.

Twenty pearl millet cultivars, identified from more than 4,000 germ plasm accessions as relatively less susceptible to ergot, were intermated, and the progenies of these crosses were screened for ergot resistance at each generation from F2 to F6, using an improved resistance screening technique. At F<sub>2</sub>, no population had a mean ergot severity of less than 20%, but when individual inflorescences that had little or no ergot were selected at each generation to provide selfed seed for the next generation, resistance levels increased steadily. At F6, 27 of 98 lines had no more than 1% mean ergot severity, an additional 29 F<sub>6</sub> lines had mean ergot severities of between 2 and 10%, and susceptible checks showed 76-95% severity. This assembly of resistance factors is indicative of the rich genetic diversity in the pearl millet germ plasm collection and of the potential for selecting increased levels of certain characters by appropriate screening and selection techniques.

Additional key words: Pennisetum americanum, Claviceps fusiformis.

Ergot, caused by Claviceps fusiformis Loveless, was a minor disease of pearl millet (Pennisetum americanum (L.) Leeke) in India until the early 1970s when it became a serious problem in crops of the recently developed and adopted commercial F<sub>1</sub> hybrids (1,7-10). Today, ergot is one of the principal factors preventing the realization of the high grain-yield potential of pearl millet hybrids in Asia and Africa. In addition, it reduces the quality of the grain in an infected crop by contaminating it with the alkaloid-containing sclerotia of the pathogen (2,3,5,6). Thakur and Williams (10) presented evidence of a negative relationship between pollen availability and ergot severity in pearl millet and suggested that F1 hybrids were more vulnerable to ergot than traditional openpollinated varieties due to several factors that reduce pollen availability in hybrids at the critical time for infection. This understanding of the role of pollination in ergot-escape in pearl millet led to the development of an improved resistance screening technique. This article describes the technique and the progress made in its large-scale use in developing pearl millet lines with a high degree of ergot resistance.

# MATERIALS AND METHODS

Initial screening techniques. During the 1975 and 1976 monsoon (rainy) seasons and the 1976-77 dry (postrainy) season, pearl millet germ plasm accessions were screened in the field at the farm of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/04040603/\$03.00/0 ©1982 The American Phytopathological Society

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) near Hyderabad, India, by dipping pearl millet inflorescences into aqueous conidial suspensions made with fresh honeydew conidia from infected plants. Conidial concentrations were standardized at about 1 × 10<sup>6</sup> conidia per milliliter. Inoculated inflorescences were covered with brown paper bags, and ergot ratings were made 14-28 days after inoculation. In 1975 the ratings were a direct assessment of the proportion of florets infected; in 1976 and early 1977 the inflorescences were scored on a 1-5 scale in which 1 was no ergot and 5 was more than 20% ergot. The class scores were used to calculate percent infection indices by the following formula:  $[(X_2 + 2X_3 + 3X_4 + 4X_5)/4N] \times 100$ , where X<sub>2</sub>-X<sub>5</sub> are the numbers of inflorescences in classes 2-5, respectively, and N is the total number of inflorescences examined. At the same time that the initial screening for resistance was being conducted, several factors thought to affect the success of inoculation were examined (4,10). The results of these latter studies led to the development of the improved resistance screening technique, which was used to screen F2 and succeeding generations, testing two crops each year since the 1977 rainy season.

Improved screening technique. Planting of test material was timed so that crop flowering occurred during the period with highest probability of cool wet weather during the rainy season and cool weather during the postrainy season (based on analysis of 70 yr of daily weather records by ICRISAT agroclimatologists). The main shoots of all plants to be inoculated were bagged with semiopaque white parchment bags at the "boot-leaf" stage, with the bags stapled tightly around the stems at the base of the "boots," so that the inflorescences emerged into a pollen-free and inoculumfree environment. When the bagged inflorescences were at the full fresh-stigma stage, the bags were briefly removed and the inflorescences were inoculated by spraying to runoff with aqueous conidial suspensions (about 1 × 10<sup>6</sup> conidia per milliliter), derived from highly susceptible, infected inoculum-source plants. The bags were replaced immediately after inoculation to minimize possible contamination of the inflorescences with external pollen. Overhead sprinkler irrigation was used for three 30-min periods on rainfree days throughout the inoculation and disease-development period during the rainy season and daily during the postrainy season screening. Ten to 14 days after inoculation, the proportion of ergot-infected florets was estimated and recorded for each inoculated inflorescence, with the aid of standard drawings previously described (10). Single rows of highly ergot-susceptible lines, planted between every 10 test plots throughout the screening area each season, were similarly inoculated to provide a measure of success of the screening process.

Test materials, hybridization, and selection. More than 4,000 accessions from the ICRISAT pearl millet germ plasm collection were screened in single 4-m rows during the 1975 and 1976 rainy seasons and the 1976–1977 postrainy season. Most accessions developed high levels of ergot and none was consistently resistant.

TABLE 1. Percent ergot infection indices<sup>a</sup> of 20 pearl millet lines screened for ergot reactions in 1975<sup>b</sup>, 1976, and 1977 and used as parents in a program to develop ergot resistance

	Yea	r
Line <sup>c</sup>	1976	1977
J 606-2	11	23
J 703-1	5	57
J 797-1	12	25
J 1553	0	44
J 1999	7	31
J 2210-2	7 3	14
J 2238	4	21
700142	4	21
700583	12	36
700599	0	19
700619	6	27
MPP 7135-3-1	12	32
IP 1926	18	32
IP 1941	0	32
IP 2253	0 2 7	52
SC-1 (S4)27-2		40
SC-1 (S4)27-3	9	35
SC-2(M)13-4	13	41
Ex Bouchi-700638-3-2	0	25
ND 2282-79-1	1	45
Susceptible check (5141A)	66	97

<sup>&</sup>lt;sup>a</sup> Percent ergot infection index =  $[(X_2 + 2X_3 + 3X_4 + 4X_5)/4N] \times 100$ , in which  $X_2 - X_5$  are the number of inflorescences in infection categories 2-5, respectively, and N is the total number of inflorescences examined. <sup>b</sup> All lines were ergot-free in 1975.

However, 20 accessions (Table 1) appeared to be consistently less susceptible than others, and these were crossed in pairs, some pairs reciprocally, to provide 269  $F_1$  progenies. The  $F_1$  plants were grown in the 1977–1978 postrainy season, and the resulting  $F_2$  populations were screened during the 1978 rainy season. Seed from individual inflorescences with little or no ergot, selected from the inoculated  $F_2$  plants, were used to produce  $F_3$  lines that were screened during the 1978–79 postrainy season. The process was repeated at  $F_4$  (rainy season 1979),  $F_5$  (postrainy season 1979–1980), and  $F_6$  (rainy season 1980), with only ergot-free inflorescences selected at the latter two generations. Additional relevant details of the screening process are provided in Table 2.

# RESULTS

The mean ergot reactions of all lines screened at each generation are summarized in Table 3. The overall levels of ergot declined steadily at each generation from  $F_2$  (no population with less than 20% mean ergot severity) to  $F_6$  (about 70% of the lines with no more than 10% ergot). A similar decline occurred in the progeny from the two crosses that contributed the most resistance among the  $F_6$  lines (Tables 4 and 5). No line was completely free from ergot at any generation, and considerable variability occurred in the range of reactions among the inflorescences within lines, even at  $F_5$ . But at  $F_6$ , many lines with very little variability (0–1% ergot severity) were identified (Table 6). Ergot severities (76–95%) in the susceptible checks (Table 7) indicate the efficiency of the screening technique.

## DISCUSSION

Through a process of intermating relatively less susceptible cultivars and screening each generation from  $F_2$  to  $F_6$  with high inoculum and selection pressures, pearl millet lines with a high degree of ergot resistance were developed. The steady increase in resistant segregants at each generation, the continued occurrence of some highly susceptible plants, and highly susceptible  $F_1$  plants from crosses between ergot-susceptible and resistant lines (R. P. Thakur and R. J. Williams, unpublished) indicate that resistance to ergot is recessive and polygenically controlled.

The absence of high levels of resistance in germ plasm accessions and the general unimportance of ergot in the regions from which they originate are consistent with the hypothesis that the variable pearl millet landrace cultivars escape ergot and thus have not been subjected to selection pressure for the assembly of genes contributing to ergot resistance into a single genome. The pollen-based escape mechanism recently described (10) and photoperiod sensitivity that often delays flowering till the rains diminish (when the environmental conditions are less conducive to ergot infection) are likely to be the major components of the ergot-escape phenomenon.

Pearl millet germ plasm is characterized by rich diversity for many evident and cryptic characters, and rapid progress can be made in assembling the required genes to strengthen or diminish these characters, provided appropriate screening and selection techniques are used. In the rapid and significant progress reported in this paper, the key factors were the availability of a large germ plasm collection, the development of an effective screening technique, and the availability of resources to enable the technique

TABLE 2. Numbers of lines screened, crosses represented, replications made, and inflorescences inoculated and selected in  $F_2$ - $F_6$  generations in a program to develop high levels of ergot resistance in pearl millet.

Lines Generation screened			Inflorescen			
		Crosses represented	Replications per line	Minimum per line	Total per generation	Inflorescences selected
F <sub>2</sub>	269	53	a	150	40,350	657
F <sub>3</sub>	657	49	•••	40	26,280	472
F4	472	18	***	40	18,880	220
Fs.	220	11	3	180	39,600	98
36	572	6	2	80-160	15,680	2,667

<sup>&</sup>lt;sup>a</sup>Single plots per entry; 10 rows per plot at F<sub>2</sub> and two rows per plot at F<sub>3</sub> and F<sub>4</sub>.

Corigin of lines: J = Jamnagar (India), 700... = breeding lines from Kano (Nigeria), MPP... = Mycology and Plant Pathology selection at New Delhi (India), IP... = world collection accession, SC... = Serere composite-breeders population (Uganda), Ex Bouchi = from Bouchi (Nigeria), ND... = New Delhi (India).

TABLE 3. Percentage<sup>a</sup> of pearl millet lines<sup>b</sup> in seven ergot severity classes in five generations in which the seed for the succeeding generation was taken from the least infected plants in the preceding generation

Mean ergot severity (%)	Percentage of lines in each class at					
	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>	F	
<1	0	0	0	1	14	
1-10	0	2	15	14	56	
11-20	0	6	21	19	18	
21-30	6	11	28	16	9	
31-40	9	16	22	20	2	
41-50	18	16	9	15	<1	
>50	67	49	6	16	0	

<sup>\*</sup>Rounded off to whole numbers.

TABLE 4. Seven ergot severity classes in five generations of pearl millet from a cross<sup>a</sup> between J 2238 and J 2210-2

Mean ergot severity (%)	Percentage <sup>b</sup> of lines in each class at						
	F2°	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>	F <sub>6</sub>		
<1	0	0	0	4	31		
1-10	0	0	63	36	59		
11-20	0	7	21	24	5		
21-30	0	16	16	4	0		
31-40	33	36	0	13	3		
41-50	33	10	0	2	0		
>50	0	32	0	16	2		
Total lines screened	3	31	19	45	64		

<sup>&</sup>lt;sup>a</sup>One of two crosses having the greatest resistance at F<sub>6</sub>.

TABLE 5. Seven ergot severity classes in five generations of pearl millet from a cross<sup>a</sup> between J 606-2 and J 703-1

Mean ergot severity (%)	Percentage <sup>b</sup> of lines in each class at						
	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>	F <sub>6</sub>		
<1	¢	,0	0	9	82		
1-10		5	13	18	9		
11-20	***	5	14	27	3		
21-30	•••	16	31	9	3		
31-40		11	31	36	3		
41-50	•••	21	7	0	0		
>50	***	42	4	0	0		
Total lines screened	6	19	71	11	34		

One of two crosses having the greatest resistance at F6.

to be used intensively on a large scale over several seasons. The development of the ergot-resistant lines described is, however, only the first step. If the resistance is to be of use, it will have to be

TABLE 6. Variability of pearl millet F6 lines from two crosses

Cross	No. of lines with maximum ergot severity (%) on any individual inflorescence of						
	0	≤1	2-10	11-20	>20		
J 2238 × J 2210-2	0	7	22	6	29		
J 606-2 × J 703-1	0	20	7	2	5		

TABLE 7. Ergot reactions (%) of susceptible checks planted in every 11th plot throughout the ergot-screening nurseries

Season		Susceptible checks				
	Generation	514	1-A	BJ-104		
	screened	Mean	Range	Mean	Range	
1978 rainy	F <sub>2</sub>	95	60-100	81	60-95	
1978-1979 postrainy	F <sub>3</sub>	95	50-100	90	75-100	
1979 rainy	F <sub>4</sub>	85	50-100	91	70-100	
1979-1980 postrainy	F <sub>5</sub>	76	30-98	90	50-98	
1980 rainy	$F_6$	85	75-98	94	75-100	

combined with resistance to downy mildew and smut, two other diseases of major importance in pearl millet, and it must be incorporated into parents for the production of  $F_1$  hybrids. The ICRISAT pearl millet improvement team is working intensively on these aspects and will make available to any scientist on request small quantities of the newly developed ergot-resistant lines described.

### LITERATURE CITED

- Arya, H. C., and Kumar, A. 1976. Diseases of Bajra—A serious problem of Rajasthan desert economy. Trans. Indian Soc. Desert Technol. and Univ. Center Desert Sci. 1:177-182.
- Bhat, R. V., Roy, D. N., and Tulpule, P. G. 1976. The nature of alkaloids of ergoty pearl millet or bajra and its comparison with alkaloids of ergoty rye and ergoty wheat. Toxicol. Appl. Pharmacol. 36:11-17.
- Bhide, N. K., and Sheth, U. K. 1957. Pharmacological study of infected bajra seed. Indian J. Med. Sci. 11:892-895.
- ICRISAT. 1977. Pearl millet pathology. ICRISAT Annual Report, 1976–1977. Int. Crops Res. Inst. Semi-Arid Tropics, Hyderabad, India.
- Kannaiyan, J., Vidhyasekaran, P., and Kandaswamy, T. K. 1971.
   Mammalian toxicity of ergot of bajra. Curr. Sci. (Bangalore) 40:557-558.
- Krishnamachari, K. A. V. R., and Bhat, R. V. 1976. Poisoning by ergoty bajra (pearl millet) in man. Indian J. Med. Res. 64:1624-1628.
- Ramakrishnan, T. S. 1971. Diseases of Millets. Indian Counc. Agric. Res., New Delhi. 152 pp.
- Safeeulla, K. M. 1977. Genetic vulnerability: The basis of recent epidemics in India. I. Pages 72-85 in: P. R. Day, ed. The Genetic Basis of Epidemics in Agriculture. Ann. N.Y. Acad. Sci. Vol. 287.
- Sundaram, N. V. 1975. Ergot of bajra. Pages 155-160 in: Advances in Mycology and Plant Pathology. Prof. R. N. Tandon's Birthday Celebration Committee, New Delhi. 343 pp.
- Thakur, R. P., and Williams, R. J. 1980. Pollination effects on pearl millet ergot. Phytopathology 70:80-84.

<sup>&</sup>lt;sup>b</sup>The F<sub>2</sub> lines were from 53 crosses and the F<sub>6</sub> progeny were from only six of these crosses.

<sup>&</sup>lt;sup>b</sup>Rounded off to whole numbers.

<sup>&</sup>lt;sup>c</sup>Three populations screened but only two scored.

<sup>&</sup>lt;sup>b</sup>Rounded off to whole numbers.

<sup>&</sup>lt;sup>c</sup>Ergot reactions were not recorded, but ergot-free inflorescences were selected for the next generation.