# Ergot Susceptibility in Relation to Cytoplasmic Male Sterility in Pearl Millet

R. P. THAKUR, Senior Plant Pathologist, V. P. RAO, Research Associate, and S. B. KING, Principal Plant Pathologist, Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P. 502 324, India

**ABSTRACT**

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The association of cytoplasmic male sterility with susceptibility to ergot (caused by *Claviceps fusiformis*) in pearl millet was studied in seven cytoplasmic male-sterile (CMS) lines (A lines), their corresponding maintainer lines (B lines), 10 pollinator lines (R lines), and 48 $F_1$ hybrids (A × R and B × R). Four of the seven A lines were significantly more susceptible (90–97% severity) than their corresponding B lines (54–77% severity). All A × R hybrids except four were significantly more susceptible than their corresponding B × R hybrids, indicating the positive association of sterile cytoplasm with ergot susceptibility. The cytoplasmic effect on ergot susceptibility was significant in 20 of the 24 A/B × R hybrids. The higher ergot susceptibility of A lines and A × R hybrids, compared with B lines and B × R hybrids, could be attributed to the cytoplasmic, nuclear, and cytoplasmic × nuclear factors affecting flowering events that influence ergot susceptibility. Implications of these findings in breeding CMS-based $F_1$ hybrids are discussed.

Additional keywords: *Pennisetum glaucum*

The discovery of cytoplasmic male sterility in pearl millet (*Pennisetum glaucum* (L.) R. Br.) and the development and release of Tift 23A (3,4) made possible the production of commercial $F_1$ grain hybrids in India (4,8), where more than 40% of the pearl millet in the world is grown. With the large-scale cultivation of hybrids, ergot, caused by *Claviceps fusiformis* Loveless, known to be a minor disease until the 1960s (10), became a disease of national importance in the mid-1970s. Today ergot is recognized as a major threat to the commercial cultivation of $F_1$ hybrids of cytoplasmic male-sterile (CMS) pearl millet in India (1), and grain yield loss of up to 65% has been obtained with artificial inoculation (15).

Pearl millet cultivation in Africa, where more than 50% of the world's crop is grown, is almost exclusively based on landraces, with limited use of improved varieties derived from these landraces and essentially no cultivation of hybrids. Ergot is presently not a serious problem in African countries, although the disease is endemic in many areas of cultivation. However, the potential for ergot to become a major threat in Africa is evidenced by its consistent severe appearance on experimental male-sterile lines and CMS $F_1$ hybrids at research stations.

All the currently available CMS lines (A lines), their corresponding maintainer lines (B lines), pollinator lines (R lines), and the commercial and experimental CMS hybrids made with them are highly susceptible to ergot (1,6). Generally, A lines are more susceptible than B and R lines, and open-pollinated varieties are considered to be less susceptible than $F_1$ hybrids (1,2,16,18). Ergot susceptibility in pearl millet is associated with long protogyny (18,21) and low pollen production (R. P. Thakur, *unpublished*).

Our objective in this study was to test the hypothesis that high ergot susceptibility in hybrids and parental lines of pearl millet is more closely associated with cytoplasmic male sterility than with normal cytoplasm.

## MATERIALS AND METHODS

**Pearl millet lines and $F_1$ hybrids.** Seven A lines and their corresponding B lines (852A/B, 5054A/B, 81A/B, 834A/B, 843A/B, 5141A/B, and 841A/B) and 10 R lines, including four ergot-resistant inbreds (ICMPES 1, ICMPES 2, ICMPE 134-6-9, and ICMPE 13-6-30) and six ergot-susceptible inbreds (ICMP 851009, J 104, ICMP 451, ICMP 501, ICMP 85417, and ICMP 423), were used in two different field experiments at ICRISAT Center (Tables 1 and 2). Both A and B lines of each pair were used as female parents in crosses with R lines, to produce $F_1$ hybrids.

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In experiment 1, conducted during the 1983 rainy season (July–September), three A/B pairs and two ergot-resistant lines were used; in experiment 2, conducted in the 1987 rainy season, six A/B pairs (81A/B and 5054A/B were common in both experiments) and eight R lines, including two ergot-resistant lines, were used.

Seeds for $F_1$ CMS hybrids were produced in the preceding dry season (January–April) following the conventional breeding procedure, which involves pollinating the female parent at protogyny with pollen from the male parent. During bagging and crossing, care was taken to avoid cross-contamination. Seeds from the crossed female plants were collected to grow $F_1$ hybrid plants.

**Planting and experimental design.** In both experiments, each line was planted in a one-row plot 4 m long, with the rows spaced at 75 cm, and plants were thinned to 20–25 plants per row. In experiment 1, there were 20 treatments (eight parental lines and 12 $F_1$ hybrids), and in experiment 2, there were 56 treatments (20 parents and 36 $F_1$ hybrids). Each treatment was replicated three times in a randomized block design.

**Inoculum, inoculation, and ergot scoring.** A conidial suspension of *C. fusiformis* ($1 \times 10^6$ conidia per milliliter), prepared from honeydew produced on previously inoculated panicles of a susceptible pearl millet line, was used for inoculation.

Panicles were protected from cross-pollination and other external contamination by bagging at the boot leaf stage. The bags were briefly opened at 75% protogyny to spray-inoculate panicles with a hand-held pressure sprayer until all florets were covered with the conidial suspension of *C. fusiformis* (19). In each row, 20 plants were inoculated. High humidity was provided by overhead sprinkler irrigation twice a day (1200–1300 hr and 1700–1800 hr) on rainfree days, for up to 10 days after inoculation.

The bags were removed 20 days after inoculation, and panicles were scored for ergot severity, with an assessment key of 0–100%, in which 0 indicates no ergot infection and 100 indicates infection of all the florets on a panicle (18).

**Analysis of data.** Estimates of the cytoplasmic effect on ergot susceptibility were determined by computing the differences in ergot severity levels between A and B lines and between A × R and B × R hybrids, and the data were subjected to ANOVA to determine the LSD ($p = 0.05$).

## RESULTS

**Ergot severity of A, B, and R lines.** Among the seven A lines, 834A had the lowest ergot severity (78%), and 852A, 5054A, and 843A had the highest (97%) (Tables 1 and 2). Among the B lines, 834B and 852B both had the lowest severity (54%), and 843B had the highest (92%). The severity levels of four of the seven A lines were significantly higher than those of their corresponding B lines (Tables 1 and 2). Among A/B pairs, significantly less ergot was recorded in 834A/B than in other pairs. The ergot-resistant lines used as R lines (ICMPE and ICMPES lines) were highly resistant, but the other pollinators were all susceptible, with ergot severity ranging from 34 to 92% (data are not available for J 104); ICMP 85417 was the most susceptible.

**Ergot severity of $F_1$ hybrids (A × R and B × R).** The mean ergot severities of A × R hybrids varied from 59% for 834A × R lines to 92% for 843A × R lines (Tables 1 and 2). The mean ergot severities of B × R hybrids varied from 29% for 834B × R lines (Table 2) to 71% for 5054B × R lines (Table 1).

All $F_1$ hybrids of A × R had significantly higher ergot severity than B × R hybrids, except 852A/B × ICMP 851009, 81A/B × ICMP 451, 834A/B × ICMP 501, and 843A/B × ICMP 85417 (Table 2). Among B × R hybrids, ergot severity was always significantly lower in hybrids with ergot-resistant R lines than in hybrids with ergot-susceptible R lines, but such differences were not observed in A × R hybrids (Table 2). The least ergot was recorded in 843B × ICMPES 2 (12%), followed by 834B × ICMPES 1 (15%). These levels were significantly lower ($p = 0.05$) than those recorded in other B × R hybrids

**Table 1.** Ergot reactions of cytoplasmic male-sterile (A), maintainer (B), and pollinator (R) lines of pearl millet and their hybrids in 1983, with estimated differences in ergot susceptibility associated with cytoplasm

| | Mean ergot severity (%)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Cross (A/B × R) | A | B | R | A − B | A × R | B × R | A × R − B × R |
| 5141A/B × ICMPE 134-6-9 | 90 | 77 | 1 | 13 | 69 | 52 | 17 |
| 5141A/B × ICMPE 13-6-30 | | | 1 | | 88 | 68 | 20 |
| 5054A/B × ICMPE 134-6-9 | 88 | 80 | 1 | 8 | 86 | 70 | 16 |
| 5054A/B × ICMPE 13-6-30 | | | 1 | | 93 | 72 | 21 |
| 81A/B × ICMPE 134-6-9 | 91 | 90 | 1 | 1 | 82 | 46 | 36 |
| 81A/B × ICMPE 13-6-30 | | | 1 | | 92 | 50 | 42 |
| LSD ($P = 0.05$) | 3.9 | 6.8 | 0.9 | 8.8 | 6.6 | 11.9 | 14.3 |

[a] Based on 20 inoculated panicles from each of three replications at ICRISAT Center.

**Table 2.** Ergot reactions of cytoplasmic male-sterile (A), maintainer (B), and pollinator (R) lines of pearl millet and their hybrids in 1987, with estimated differences in ergot susceptibility associated with cytoplasm

| | Mean ergot severity (%)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| Cross (A/B × R) | A | B | R | A − B | A × R | B × R | A × R − B × R |
| 852A/B × ICMPES 1 | 97 | 54 | 1 | 43 | 96 | 65 | 31 |
| 852A/B × ICMPES 2 | | | 1 | | 87 | 60 | 27 |
| 852A/B × ICMP 851009 | | | 34 | | 85 | 77 | 8 |
| 5054A/B × ICMPES 1 | 97 | 59 | 1 | 38 | 90 | 32 | 58 |
| 5054A/B × ICMPES 2 | | | 1 | | 88 | 38 | 50 |
| 5054A/B × J 104 | | | —[b] | | 86 | 55 | 31 |
| 81A/B × ICMPES 1 | 90 | 82 | 1 | 8 | 94 | 48 | 46 |
| 81A/B × ICMPES 2 | | | 1 | | 81 | 28 | 53 |
| 81A/B × ICMP 451 | | | 45 | | 85 | 72 | 13 |
| 834A/B × ICMPES 1 | 78 | 54 | 1 | 24 | 75 | 15 | 60 |
| 834A/B × ICMPES 2 | | | 1 | | 58 | 30 | 28 |
| 834A/B × ICMP 501 | | | 50 | | 45 | 43 | 2 |
| 843A/B × ICMPES 1 | 97 | 92 | 1 | 5 | 93 | 42 | 51 |
| 843A/B × ICMPES 2 | | | 1 | | 88 | 12 | 76 |
| 843A/B × ICMP 85417 | | | 92 | | 96 | 94 | 2 |
| 841A/B × ICMPES 1 | 93 | 61 | 1 | 32 | 81 | 43 | 38 |
| 841A/B × ICMPES 2 | | | 1 | | 80 | 35 | 45 |
| 841A/B × ICMP 423 | | | 69 | | 96 | 71 | 25 |
| LSD ($p = 0.05$) | 9.7 | 23.6 | 17.6 | 31.0 | 8.2 | 13.1 | 17.3 |

[a] Based on 20 inoculated panicles from each of three replications at ICRISAT Center.
[b] Data not available.

except 81B × ICMPES 2. The highest ergot severity (94%) was recorded in 843B × ICMP 85417, whose parents both had comparably high severity levels.

## DISCUSSION

The results of this study indicate a positive and significant association of ergot susceptibility with male-sterile cytoplasm in pearl millet. This was true for four of the seven A/B pairs and for 20 of the 24 A/B × R hybrids. Significant differences in ergot severity levels between A and B lines are clearly related to differences between the sterile and the normal cytoplasms, with the nuclear effects theoretically the same in both the A and the B lines. All seven CMS A lines used in this study belong to the $A_1$ sterility system based on Tift 23A (9) and are currently being used at ICRISAT Center to produce $F_1$ hybrids. These seven and 45 others from the All-India Coordinated Pearl Millet Improvement Project, which were evaluated at ICRISAT Center during 1983–1987, have shown higher susceptibility to ergot than their corresponding B lines (R. P. Thakur, *unpublished*).

Although the reasons for the greater ergot susceptibility associated with cytoplasmic male sterility are not clearly understood, previous studies (18,21) indicate that ergot resistance is based on a pollen-based escape mechanism. Once pollination and subsequent fertilization occur, susceptibility to ergot is dramatically reduced (18). Where pollination is dependent on selfing, panicles with short protogyny are much less susceptible than ones with long protogyny. The lack of significant differences in ergot severity levels in two of the A/B pairs (81A/B and 843A/B) and three $F_1$ hybrid pairs of A/B × R (5054A/B × ICMP 134-6-9, 834A/B × ICMP 501, and 843A/B × ICMP 85417) could be due to cytoplasmic × nuclear interaction effects. Ergot susceptibility in pearl millet could be due to the effect of nuclear genes, cytoplasmic genes, or their interaction (1). These would also influence protogyny length and flowering events such as pollen production, fertility restoration, and self-compatibility, and these factors directly or indirectly might influence ergot severity. Some of these factors are being studied at ICRISAT Center. Preliminary findings, based on the amount of seed set in bagged panicles, suggest that pollen fertility is significantly lower in A × R hybrids than in B × R hybrids (C. T. Hash, *personal communication*). In general, B × R hybrids had lower ergot severity than comparable A × R hybrids, and B × R hybrids with ergot-resistant R lines had lower ergot severity than those with ergot-susceptible R lines. These differences may be due to the effects of cytoplasmic × nuclear and nuclear × nuclear interactions on ergot susceptibility.

Cytoplasmic male sterility is known to be associated with increased susceptibility to ergot in wheat, caused by *C. purpurea* (7,11,14), and ergot in sorghum, caused by *Sphacelia sorghi* (5). In other diseases in which pollen is of no consequence, such as yellow leaf blight of corn, caused by *Phyllosticta zeae* (12), and southern corn leaf blight, caused by *Bipolaris maydis*, cytoplasmic male sterility has been reported as a major factor relating to increased disease susceptibility (13,20).

The implications of these findings for hybrid breeding in pearl millet are significant, and the higher susceptibility of CMS lines and hybrids to ergot is of major concern for the control of this disease. It is also evident that to produce ergot-resistant hybrids, both female and male parents should have resistance, which is genetically similar, since ergot resistance is reported to be recessive and polygenic (17). Our experience at ICRISAT Center has so far shown that it is difficult and time-consuming to transfer the existing type of resistance, which is based on short protogyny and is pollination-mediated (21), into the parental lines of an established hybrid. Other strategies that may hold more promise for breeding ergot-resistant hybrids include the production of single-cross hybrids without cytoplasmic sterility, the use of either long protogyny or chemical hybridizing agents, and breeding of top-cross hybrids, using currently available ergot-resistant lines as pollinators. Top-cross hybrids, based on pollinators with heterogeneous plant phenotypes and asynchronous tillering and flowering, would provide pollen for an extended period, as is characteristic of open-pollinated varieties that more easily escape ergot infection (19). However, fertility restoration in top-cross pearl millet hybrids is generally lower than in single-cross hybrids (J. R. Witcombe, *personal communication*).


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