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# Screening for Ergot Resistance in Sorghum

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**ABSTRACT**

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Experiments were conducted at Arsi Negele, Ethiopia, during the 1988 and 1989 rainy seasons to determine a suitable combination of sorghum panicle trimming (a method used to remove pollinated spikelets), inoculation, and bagging to develop an ergot resistance screening technique. Results showed that the most suitable method was a single inoculation of nontrimmed panicles when anthesis began in a panicle, followed by bagging. Comparison of resistance evaluation methods suggested that susceptible genotypes could be identified by a simple and rapid visual ergot rating on a 1-5 scale, where 1 = no ergot and 5 = more than 50% spikelets in a panicle infected. However, resistance of genotypes should be confirmed by counting infected and healthy spikelets in a few primary branches of panicles. Screening of 213 Ethiopian sorghum accessions led to the identification of six ergot-resistant lines—ETS 1446, 2448, 2465, 3135, 4457, and 4927—that are well adapted to the highlands of Ethiopia.

Additional keywords: *Claviceps africana*, *Sphacelia sorghi*, sugary disease

Ergot is a disease of sorghum (*Sorghum bicolor* (L.) Moench) inflorescence caused by *Claviceps africana* Frederickson, Mantle, & de Milliano in Africa. Conidia of *Sphacelia sorghi* McRae, the anamorph, infects the stigma before or at anthesis and replaces the ovary with a soft fungal mass (stroma) that is later converted into a hard sclerotium. The stroma produce numerous conidia in a sugary fluid, called honeydew, which contaminates the grains and provides a substrate for growth of saprophytic fungi (1).

The disease was first recorded on sorghum by McRae in 1917 (12) in India. It was noticed at Alemaya, Ethiopia, in experimental plots in 1982 but was not considered to be important (8). It has subsequently been reported in seven Asian and 18 African countries (4,5). At locations where the disease is present, hybrid seed production plots are particularly vulnerable to ergot damage if restorer lines do not produce adequate pollen when stigmas of male-sterile lines are receptive (1). Low night temperatures (below 12 C) commonly occur in sorghum-growing areas of the eastern and central African highlands and induce pollen sterility, thereby predisposing flowers to ergot infection (11). The disease has recently become more important in eastern and southern Africa because of the widespread use of male-sterile lines and poorly adapted parental materials in breeding high-yielding cultivars and hybrids (5). Host plant resistance is an important method of ergot control, particularly for subsistence producers of eastern Africa.

A number of artificial inoculation techniques and disease evaluation methods have been used to screen sorghum for ergot resistance in the field and greenhouse (3,9,10,16,17). The methods have varied with respect to plant growth stages at the time of inoculation, number of inoculations, and panicle bagging. There is limited evidence of reliable sources of resistance. Except for McLaren (10), published accounts of resistance have been based on results of single nonreplicated trials, and putative resistant lines have proved susceptible in subsequent tests (3,9,16,17).

To be effective, a screening technique for ergot resistance should ensure the availability of viable inoculum to sorghum pistils at the stigma before pollination, because pollinated spikelets resist infection (6). Favorable temperature and humidity for infection are also required. This paper reports the development of an effective ergot resistance screening technique and the identification of sources of resistance adapted to the high-altitude sorghum-growing areas of Ethiopia. A preliminary account of a portion of this work has been published (13).

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## MATERIALS AND METHODS

Experiments were conducted at Arsi Negele Research Station, Ethiopia, 1,960 m above sea level, where sorghum develops severe ergot infection in most years.

**Inoculum and inoculation.** A conidial suspension of *S. sorghi* was prepared by washing infected sorghum (cv. Melkamash A, i.e., male-sterile Melkamash) panicles containing fresh honeydew. The resultant suspension was filtered through two layers of cheesecloth and diluted to contain approximately $1 \times 10^6$ conidia per milliliter. Panicles were inoculated with a hand sprayer until they were soaked in the suspension and it ran off.

**Development of artificial inoculation technique.** Eight treatment combinations of spikelet trimming (trimming and no trimming), inoculation (one and two at a 2-day interval), and bagging (no bagging and bagging for 7-10 days) were compared in a $2 \times 2 \times 2$ factorial experiment during the 1988 and 1989 rainy seasons (Table 1). Spikelet trimming involved removing all the spikelets in a panicle that had completed anthesis before inoculation. Panicles were considered not to require trimming if they were inoculated when anthesis in a few spikelets had begun at the tip of the panicle. The genotypes used were ETS 3135 (resistant) and ETS 0223 and Melkamash A (highly susceptible) in 1988 and ETS 3135 and IS 9302 (susceptible) in 1989. The unit plot was two rows, each 4 m long, with four replications in 1988 and two in 1989.

To record the severity of ergot infection 30-40 days after inoculation, five panicles were harvested from each plot. In 1988, each panicle was evaluated for ergot severity, first on a 1-5 visual rating scale where 1 = no ergot, 2 = 1-10%, 3 = 11-25%, 4 = 26-50%, and 5 = more than 50% spikelets in a panicle infected, and second by counting the number of spikelets with stroma, with healthy grain, and without either in a composite sample of spikelets from one primary branch from each node of the rachis. The percentages of infected spikelets, unfilled spikelets, and spikelets without grain, i.e., infected plus unfilled spikelets, were calculated. In 1989, only the second method was used for evaluation.

**Comparison of ergot evaluation methods.** In 1988, paired data were available for 358 panicles for the visual rating and quantification of percent infected spikelets. The percent data were coded into a 1-5 rating, termed "coded ergot rating," following the same range boundaries (0, 1, 10, 25, and 50%) of the visual

ergot rating scale. The ratings based on visual and quantitative assessments were compared by a paired *t* test in two ways. Within each genotype, means of the visual and coded ergot ratings of all panicles were calculated and compared. In the second method, means of the coded ergot ratings of all panicles in each of the visual ergot rating classes (1–5) were compared with the visual ergot rating of the group.

**Screening for ergot resistance.** Ethiopian sorghum germ plasm obtained from the Plant Genetic Resources Center of Ethiopia was screened to identify ergot resistance. A preliminary screening trial in 1987 had 213 entries, each as a single 4-m row plot, spaced 0.75 cm apart, with two rows of the susceptible genotype Melkamash A sown as a control after every 20 test entries. Entries with up to 20% infected spikelets were selected and evaluated in an advanced screening trial of the same design in 1988. Entries with up to 15% infected spikelets in 1988 were tested again in 1989, using a completely randomized design with two replications. Each plot consisted of two 4-m rows.

In each plot, five to seven panicles were trimmed, inoculated, and bagged during the morning or evening hours. After 2 days, the bags were removed, panicles were reinoculated, and the bags were replaced for an additional 7–10 days. The percent infected spikelets was determined as previously described. The analysis of variance considered each panicle of an entry in preliminary and advanced screening trials as a replicate.

## RESULTS

**Artificial inoculation technique.** Ergot severity of nontrimmed and trimmed panicles was similar, and one inoculation produced as much ergot as two inoculations (Table 1). Bagged panicles had significantly more infected spikelets than nonbagged panicles of both the resistant (ETS 3135) and susceptible (IS 9302) genotypes (Tables 1 and 2). None of the interaction effects of trimming, bagging, and inoculations within genotypes were significant (Table 2). In highly susceptible genotypes (ETS 0223 and Melkamash A), there was no response to the trimming, inoculation, or bagging treatments (Table 2); ergot severity was equally high for all treatments. Similar trends were observed for percentages of unfilled spikelets and spikelets without grain (*data not presented*). The results of the experiments in 1988 and 1989 were consistent.

**Comparison of ergot evaluation methods.** The visual and quantitative (coded) ratings were similar for the highly susceptible genotypes, but the visual rating was significantly lower than the quantitative rating for the resistant genotype ETS 3135 (Table 3). The quantitative rating was significantly greater than the visual rating for panicles that were rated visually as 2, 3, and 4 but not for those rated 5 (Table 4).

**Screening for ergot resistance.** Of the 213 entries screened in 1987, 55 (with up to 20% infected spikelets) were selected and retested in 1988. Twelve entries were

**Table 1.** Effect of panicle trimming, bagging, and inoculation on severity of ergot infection in resistant (ETS 3135), susceptible (IS 9302), and highly susceptible (ETS 0223 and Melkamash A) sorghum genotypes

| Treatments | | | Infected spikelets (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1988 | | | 1989 | |
| Trimming[y] | Bagging | No. of inoculations | ETS 3135 | ETS 0223 | Melkamash A | ETS 3135 | IS 9302 |
| +[z] | + | 1 | 8.8 | 71.8 | 95.8 | 12.8 | 49.8 |
| + | + | 2 | 9.3 | 69.8 | 95.5 | 10.4 | 48.4 |
| + | − | 1 | 1.8 | 70.1 | 92.0 | 3.7 | 12.4 |
| + | − | 2 | 3.1 | 65.5 | 91.8 | 2.9 | 12.0 |
| − | + | 1 | 9.2 | 61.9 | 92.7 | 12.1 | 53.4 |
| − | + | 2 | 6.7 | 64.8 | 91.4 | 7.5 | 51.1 |
| − | − | 1 | 3.7 | 58.5 | 84.3 | 6.3 | 16.5 |
| − | − | 2 | 2.7 | 75.8 | 89.7 | 6.5 | 19.3 |
| SE ($P \leq 0.01$) to compare any two treatment levels | | | ±1.32 | ±3.33 | ±2.55 | ±1.58 | ±3.79 |

[y] All flowering spikelets were cut immediately before inoculation to facilitate inoculation of nonpollinated spikelets. In the treatment without trimming, panicles were inoculated 1 day after initiation of anthesis.
[z] + = Treated, − = nontreated.

**Table 2.** Analysis of variance for the effect of panicle trimming (T), bagging (B), and inoculation (I) on severity of ergot infection in resistant (ETS 3135), susceptible (IS 9302), and highly susceptible (ETS 0223 and Melkamash A) sorghum genotypes

| | 1988 | | | | 1989 | | |
|---|---|---|---|---|---|---|---|
| | Mean squares[x] | | | | Mean squares | | |
| Sources of variation | df | ETS 3135 | ETS 0223 | Melkamash A | df | ETS 3135 | IS 9302 |
| T[y] | 1 | 0.32 | 129.61 | 142.81 | 1 | 1.63 | 78.32 |
| Error A | 3 | 3.46 | 100.06 | 20.97 | 1 | 3.90 | 184.96 |
| I | 1 | 1.44 | 92.48 | 6.48 | 1 | 15.02 | 0.42 |
| T × I | 1 | 14.04 | 359.12 | 10.12 | 1 | 0.39 | 1.32 |
| Error B | 6 | 6.25 | 128.72 | 24.22 | 2 | 3.52 | 42.25 |
| B | 1 | 257.64*** | 0.98 | 153.12 | 1 | 136.31** | 5,069.44*** |
| T × B | 1 | 6.84 | 92.48 | 3.38 | 1 | 23.77 | 6.76 |
| B × I | 1 | 2.88 | 68.44 | 23.80 | 1 | 10.40 | 9.61 |
| T × I × B | 1 | 0.32 | 142.81 | 20.48 | 1 | 2.48 | 4.00 |
| Error C | 12 | 4.18 | 70.47 | 39.20 | 4 | 6.36 | 4.24 |

[x] Levels of significance: ** = $P = 0.01$, *** = $P < 0.001$.
[y] All flowering spikelets were cut immediately before inoculation to facilitate inoculation of nonpollinated spikelets. In the treatment without trimming, panicles were inoculated 1 day after initiation of anthesis.

**Table 3.** Range and mean of visual ergot ratings and coded ergot ratings of resistant (ETS 3135) and highly susceptible (ETS 0223 and Melkamash A) sorghum genotypes

| Ratings | ETS 3135 | ETS 0223 | Melkamash A |
|---|---|---|---|
| Range | | | |
| Visual[x] | 2–3 | 3–5 | 5–5 |
| Coded[y] | 2–4 | 2–5 | 4–5 |
| Mean | | | |
| Visual | 2.11 a[z] | 4.70 a | 4.96 a |
| Coded | 2.58 b | 4.88 a | 5.00 a |

[x] Based on a 1–5 rating scale where 1 = no ergot and 5 = more than 50% spikelets infected in a panicle.
[y] Data on the percentage of ergot-infected spikelets were converted into coded ergot rating following the same range boundaries of the visual ergot rating scale.
[z] Numbers in the same column followed by the same letter are not significantly different as determined by paired *t* test ($P \leq 0.05$, 118 df).

**Table 4.** Mean and range of coded ergot ratings of sorghum panicles with visual ergot ratings of 2, 3, 4, and 5

| | Coded ergot ratings[y] | |
|---|---|---|
| Visual ergot rating[x] | Mean | Range |
| 2.0 a[z] | 2.52 c | 2–4 |
| 3.0 a | 3.50 b | 2–5 |
| 4.0 a | 4.62 b | 3–5 |
| 5.0 a | 4.99 a | 4–5 |

[x] Based on a 1–5 rating scale where 1 = no ergot and 5 = more than 50% spikelets infected in a panicle.
[y] Data on the percentage of ergot-infected spikelets were converted into coded ergot rating following the same range boundaries of the visual ergot rating scale.
[z] Numbers in the same row followed by the same letter are not significantly different as determined by paired *t* test ($P \leq 0.05$, 105 df for visual ergot rating 2.0, 19 df for 3.0, 20 df for 4.0, and 210 df for 5.0).

**Table 5.** Pedigree, origin, race, grain characteristics, time to 50% flowering in days (DTF), and percent ergot-infected spikelets of Ethiopian sorghum accessions resistant to ergot in field screening at Arsi Negele, Ethiopia

| Genotype | Pedigree | Origin[t] | Grain characteristics | | | DTF | Infected spikelets (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Race[u] | Color | Testa[v] | | 1987[w] | 1988[w] | 1989[x] |
| Resistant accessions | | | | | | | | | |
| ETS 1446 | WS 1584 | NA[y] | D | Brown | P | 133 | 2.7 | 2.7 | 8.7 |
| ETS 2448 | NA | Woldo | D | White | A | 125 | 17.3 | 3.5 | 14.1 |
| ETS 2465 | NA | Woldo | D | Red | A | 133 | 6.5 | -[z] | 7.0 |
| ETS 3135 | Mogne Aybelash | Shewa | DB | Red | A | 134 | 0.7 | 2.9 | 11.6 |
| ETS 4457 | Abat Beyan | Shewa | D | Red | A | 141 | 6.0 | [z] | 13.2 |
| ETS 4927 | Misc. 8 | Hararghe | DC | Brown | P | 123 | 3.0 | 2.5 | 2.6 |
| Susceptible controls | | | | | | | | | |
| ETS 4567 | | | | | | 144 | NA | NA | 100.0 |
| ETS 2113 | | | | | | 144 | NA | NA | 99.5 |
| Local cultivar | | | | | | 135 | 92.5 | NA | NA |
| 85 PGRC/E Acc. No. 137 | | | | | | 140 | 82.0 | NA | NA |
| ETS 0223 | | | | | | 140 | NA | 77.2 | NA |
| SE | | | | | | | ±1.46 | ±0.95 | ±1.43 |

[t] Ethiopian administrative regions where accessions were originally collected.
[u] D = durra, DB = durra-bicolor, and DC = durra-caudatum, according to the classification of Harlan and de Wet (7).
[v] P = present, A = absent.
[w] Mean of five inoculated panicles.
[x] Mean of 20 inoculated panicles.
[y] NA = data not available.
[z] Data on the percentage of infected spikelets not available. Visual rating of inoculated panicles (on a 1 5 scale where 1 = no ergot and 5 = more than 50% spikelets infected in a panicle) was 2, i.e., up to 10% spikelets infected in a panicle.

further selected for testing in 1989, and six entries showed <20% ergot consistently in the three screenings (Table 5). The susceptible checks had more than 90% infected spikelets. All resistant accessions were durra-based but had varying grain color.

## DISCUSSION

Six ergot-resistant sorghum genotypes were identified in a high-altitude area of Ethiopia using a new resistance screening technique. A single inoculation when anthesis began at the tip of the panicles, followed by bagging, was the most appropriate inoculation technique with respect to convenience, biological significance, and statistical inference.

Trimming pollinated spikelets before inoculation ensured that only nonpollinated spikelets at a susceptible stage were inoculated. However, trimmed and nontrimmed panicles had similar ergot severity (Table 2), probably because less than 3% spikelets in a panicle were pollinated on the first day of anthesis (15), when panicles were inoculated in this experiment. By contrast, results from similar experiments conducted in Rwanda (14) showed that pollinated spikelets should be trimmed out of the panicles inoculated more than 1 day after initiation of anthesis because about 10-25% of spikelets shed pollen and become resistant (6,14) by then. Several reports of screening techniques used for ergot resistance exist but do not indicate the rationale of the test or its reproducibility. These tests included single (16), double (17), and multiple (9) inoculations, with (16,17) or without (3,9) bagging, and visual (17) and quantitative (3,16) evaluation methods.

Both visual and quantitative ergot rating methods can be used to evaluate resistance. A visual ergot rating of 5 corresponded well with the quantitative ergot rating. However, visual ergot ratings in the range of 2-4 underestimated ergot severity, probably because some infected spikelets were not visible on the single plane of the panicle or because stromata and sclerotia were concealed inside the glumes (2). Despite a tendency to underestimation, a visual rating can be used to rapidly reject susceptible lines in large resistance screening trials (17). Quantitative evaluation is tedious and time-consuming but is appropriate for experiments in which more accurate data are required.

Unlike the testings by Chinnadurai et al (3), Sundaram (16,17), and Khadke et al (9), the ergot resistance in the six Ethiopian lines was demonstrated in repeated tests. McLaren (10) also reported several ergot-resistant genotypes in South Africa, using a regression approach to analyze ergot severity data from repeated screening trials in a range of environments (which differed primarily in temperature) and disease potentials created by adjusting planting dates. Accurate weather data for Arsi Negele are not available, but the night temperature at a nearby location (Awasa) ranged from 8 to 12 C during the preflowering period. This low temperature range is known to predispose spikelets to ergot infection (11) and is lower than the temperature range in screening trials conducted by McLaren (10).

The resistant lines reported here are adapted and have high yield potential in highland areas of Ethiopia. They are photoperiod-sensitive, and phenological conversion will be necessary if they are to be used in other parts of the world. The inheritance and mechanism(s) of resistance of these lines remain to be studied.

Seed samples of the six ergot-resistant sorghum genotypes may be obtained from the Plant Genetic Resources Center of Ethiopia, Addis Ababa.


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### LITERATURE CITED


1. Bandyopadhyay, R. 1992. Sorghum ergot. Pages 235-244 in: Sorghum and Millets Diseases: A Second World Review. W. A. J. de Milliano, R. A. Frederiksen, and G. D. Bengston, eds. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.
2. Bandyopadhyay, R., Mughogho, L. K., Manohar, S. K., and Satyanarayana, M. V. 1990. Stroma development, honeydew formation, and conidial production in *Claviceps sorghi*. Phytopathology 80:812-818.
3. Chinnadurai, G., Lalithakumari, D., and Govindaswamy, C. V. 1970. Reaction of various species and varieties of sorghum to sugary disease. Madras Agric. J. 57:735-736.
4. Commonwealth Agricultural Bureau International. 1987. Map 582 in: Commonwealth Mycological Institute Distribution Maps of Plant Diseases. Kew, England.
5. de Milliano, W. A. J., Tavares Nogueira, M. F. R., Pomela, L. M., Msiska, F. S., Kunene,

S., Matalaote, B., Mbwaga, A. M., Kaula, G. M., and Mtisi, E. 1991. New records of ergot of sorghum caused by *Sphacelia sorghi* in southern Africa. Plant Dis. 75:215.
6. Futrell, M. C., and Webster, O. J. 1965. Ergot infection and sterility in grain sorghum. Plant Dis. Rep. 49:680-683.
7. Harlan, J. R., and de Wet, J. M. J. 1972. A simplified classification of cultivated sorghum. Crop Sci. 12:172-176.
8. Hulluka, M. 1982. Diseases of sorghum at some locations in Ethiopia. Ethiop. J. Agric. Sci. 4:45-51.
9. Khadke, V. D., More, B. B., and Kone, B. K. 1978. Note on screening of sorghum varieties and selections against sugary disease. Indian J. Agric. Res. 12:257-258.
10. McLaren, N. W. 1992. Quantifying resistance of sorghum genotypes to the sugary disease pathogen (*Claviceps africana*). Plant Dis. 76:986-988.
11. McLaren, N. W., and Wehner, F. C. 1992. Preflowering low temperature predisposition of sorghum to sugary disease (*Claviceps africana*). J. Phytopathol. 135:328-334.
12. McRae, W. 1917. Notes on South Indian fungi. Pages 108-111 in: Madras Agric. Yearb.
13. Mulatu, T., and Tegegne, G. 1989. Screening sorghum varieties for ergot (*Sphacelia sorghi*) resistance. Inst. Agric. Res. Newsl. 4:7-8.
14. Musabyimana, T., Sehene, C., and Bandyopadhyay, R. Development of a screening method for ergot resistance in sorghum. Plant Pathol. In press.
15. Stephens, J. C., and Quinby, J. R. 1934. Anthesis, pollination, and fertilization in sorghum. J. Agric. Res. 49:123-136.
16. Sundaram, N. V. 1970. Sugary disease of sorghum. Pages 435-439 in: Plant Disease Problems. Proc. Int. Symp. Plant Pathol. 1st.
17. Sundaram, N. V. 1971. Possible resistance to sugary disease in sorghum. Indian J. Genet. Plant Breed. 31:383-387.