

## Ergot reaction of pearl millet hybrids affected by fertility restoration and genetic resistance of parental lines \*

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Received 13 December 1994; accepted 7 April 1995

**Key words:** *Pennisetum glaucum*, *Claviceps fusiformis*, cytoplasmic-nuclear male sterility, genetic resistance, pearl millet, ergot

### Summary

High ergot (*Claviceps fusiformis* Loveless) susceptibility of pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrids has often been associated with the A<sub>1</sub> cytoplasm of male-sterile lines (A-lines). To understand the underlying basis of this association and to examine the prospects of breeding ergot-resistant hybrids, we evaluated 56 hybrids and their 15 parental lines for ergot reaction and selfed seedset for 2 years in disease nurseries at ICRISAT Asia Center. Hybrids were made by crossing seven pollen parents (2 susceptible and 5 resistant) onto two resistant and two susceptible A-lines, and their four corresponding maintainer lines (B-lines). A-lines had no selfed seedset while B-lines had 32–75% selfed seedset. Hybrids of A-lines had significantly less selfed seedset than the hybrids of the corresponding B-lines. The reduced seedset of A-lines and their hybrids, however, was not always accompanied by significantly higher ergot susceptibility. Highly resistant hybrids were obtained where both A-lines and pollen parents were highly resistant, regardless of male fertility levels of the hybrids. Thus, although the A<sub>1</sub> cytoplasm, by its reduction of male fertility, had a large and significant effect in increasing ergot severity of hybrids, the contribution of nuclear genetic factors of female parents was about 1.8 times larger than that of the cytoplasm.

### Introduction

Ergot of pearl millet [*Pennisetum glaucum* (L.) R. Br.], caused by *Claviceps fusiformis* Loveless, is an important panicle disease in most of the semi-arid regions of Africa and the Indian subcontinent (Rachie & Majmudar, 1980; Williams, 1983). All commercial hybrids of this crop developed so far are based on male-sterile lines possessing the Tift 23A<sub>1</sub> source of cytoplasmic-nuclear male-sterility (CMS). Sporadic outbreaks of ergot reported on hybrids based on this CMS source (Natarajan et al., 1974) and higher ergot susceptibility of hybrids than of open-pollinated varieties (Thakur et al., 1983b) led to speculation regarding the association between this cytoplasm and ergot susceptibility. An investigation designed to test this hypothesis showed that male-sterile lines (A-lines) and

their hybrids were significantly more susceptible to ergot than were the corresponding maintainer lines (B-lines) and their hybrids (Thakur et al., 1989b). A later study (Thakur et al., 1991) confirmed this finding and also showed that greater susceptibility of A-lines and their hybrids than the corresponding B-lines and their hybrids might be largely due to higher degrees of male-sterility, longer protogyny, and longer periods between full protogyny and anther emergence of A-lines and their hybrids.

Ergot resistance is controlled by polygenic recessive genes, implying that to breed ergot-resistant hybrids, resistance should be incorporated in both male-sterile lines and pollen parents (Thakur et al., 1983a, 1989b). It is clear that both pollen fertility restoration of hybrids and ergot resistance of their parental lines influence ergot reaction of hybrids but their relative importance is not clear at present. Pollen parents with varying ergot-susceptibility levels have

\* Submitted as JA No. 1776 by the International Crops Research Institute for the Semi-Arid Tropics.

been available for quite some time. However, only recently have we been able to develop several ergot-resistant male-sterile lines with varying resistance levels. The objective of this research was to assess the relative roles of ergot resistance of parental lines and fertility restoration ability of pollen parents on the ergot reaction of hybrids.

## Materials and methods

### *Parental lines and hybrids*

The experimental material consisted of eight seed parents, seven pollen parents (Table 1) and 56 hybrids produced by crossing all pollen parents onto each seed parent. The eight seed parents included two susceptible A/B pairs (81A/81B and 843A/843B) and two resistant A/B pairs (ICMA 91113/ICMB 91113 and ICMA 91115/ICMB 91115). ICMB 91113 and ICMB 91115 were derived from crosses 843B × ICMER 92 and ICMER 98 × (843B × ICMER 98), respectively. The pollen parents included two potentially susceptible inbred line (ICMR 356 and H 77/833-2) that are restorers of released hybrids, and five resistant lines (ICMER 66, ICMER 92, ICMER 97, ICMER 98, and ICMER 127).

### *Field tests*

The 71 entry trial, consisting of 56 hybrids and 15 hybrid parents, was planted in a randomized complete block design in three replications in the ergot nursery at ICRISAT Asia Center (IAC) during the 1991 and 1992 rainy seasons. Each plot of one 4 m row had generally about 30 plants. At the full protogyny stage (> 75% stigma emergence), 10 panicles (1 panicle/plant) were spray-inoculated with an aqueous conidial suspension produced from honeydew of *C. fusiformis* following Thakur et al. (1982). These panicles were covered with parchment paper selfing bags, both before and after inoculation, to protect them from pollination with external pollen. Throughout the inoculation period of about two weeks, high relative humidity (> 80%) was maintained by sprinkler irrigation twice a day. Twenty days after inoculation, panicles were scored for ergot severity using an assessment key (Thakur & Williams, 1980). Five non-inoculated panicles (1 panicle/plant), bagged at the beginning of the panicle emergence in each plot, were scored for selfed seedset using the same ergot rating scale. For this, the percent surface area of

a panicle with seedset was taken as equivalent to that with ergot while the percent surface area without seedset was taken as equivalent to that with no ergot.

Five non-inoculated panicles (1 panicle/plant) in each plot of both resistant A/B pairs, three resistant pollen parents (ICMER 92, ICMER 97, and ICMER 98), and 12 hybrids were evaluated for protogyny length (time from initial stigma emergence to initial anther emergence).

### *Statistical analysis*

Data from seed parents, pollen parents and hybrids were analyzed as three different subsets. Since the error mean squares for two years were homogeneous for both ergot severity and seedset, pooled analysis over years was done for both characters. Due to poor plant stand in parental lines, seedset data could not be recorded from 12 of the 90 plots across the three replications and two years. Thus we did not attempt analysis of variance for seedset of parental lines.

Percent ergot severity and percent seedset of hybrids were analyzed using a Genstat computer program (Genstat, 1986) and following a mixed-model analysis of variance, assuming cytoplasm effects as fixed and other effects as random. Degrees of freedom and mean squares due to seed parents and their interaction with pollen parents and year were further partitioned. However, we present only those components that are relevant to the objectives of this research (i.e., cytoplasm effect, nuclear genetic resistance effect, and cytoplasm × nuclear genetic resistance interaction). Protogyny length data were analyzed assuming both male and female effects fixed.

## Results

### *Ergot severity of parental lines*

Ergot severity differences among lines in both seed parents (A/B lines) and pollen parents were highly significant ( $P < 0.01$ ). Ergot severity in susceptible seed parents (81A/81B and 843A/843B) varied from 60 to 88%, while in the resistant seed parents it was significantly less (1–26%) (Table 1). The two resistant seed parents – ICMB 91113 with 8% ergot severity and ICMB 91115 with 1% severity – were as highly resistant as their resistance donor parents ICMER 92 (5% severity) and ICMER 98 (1% severity).

Table 1. Ergot severity and selfed seedset in ergot-susceptible (ES) and ergot-resistant (ER) hybrid parents. Mean of 1991 and 1992 rainy seasons

Parental line	Pedigree/description	Ergot severity (%)	Selfed seedset (%)
Seed parents			
81A	Medium-maturity ES male-sterile line of commercial hybrids	67	0
81B	Maintainer of 81A	60	63
843A	Early-maturity ES male-sterile line of commercial hybrids	88	0
843B	Maintainer of 843A	84	32
ICMA 91113	ER male-sterile line	26	0
ICMB 91113	Maintainer of ICMA 91113 derived from 843B × ICMER 92	8	59
ICMA 91115	ER male-sterile line	8	0
ICMB 91115	Maintainer of ICMA 91115 derived from [ICMER 98 × (843B × ICMER 98)]	1	75
SE		± 4.2 <sup>1</sup>	–
Pollen parents			
ICMR 356	ES restorer line of released hybrid ICMH 356	88	46
H 77/833-2	ES restorer line of released hybrid HHB 67	26	56
ICMER 66	(J 2238 × J 2210-2)-3-3-4-6	2	59
ICMER 92	(700708-1-E-1 × J 797-1-E-1-2)-1-4	5	57
ICMER 97	[(J 606-2 × J 703-1)-4-4-5-6 × (700619 × 700599)-3-2-11-5]++	13	79
ICMER 98	[(J 2238 × J 2210-2)-3-3-4-6 × (700619 × 700599)-3-2-11-5]++	1	79
ICMER 127	[(J 2238 × J 2210-2)-3-3-4-6 × (700619 × 700599)-3-2-11-5]-2-2-1-4-2	17	66
SE		± 3.72 <sup>1</sup>	–

<sup>1</sup> SE for comparing the difference among the mean ergot severity of parental lines.

A-lines had higher ergot severity than their corresponding B-lines in all four A/B pairs. However, the differences were not statistically significant either in the two highly susceptible pairs (81A/81B and 843A/843B) or in a highly resistant pair (ICMA 91115/ICMB 91115) that had < 10% ergot severity.

The five resistant pollen parents had 1–17% ergot severity whereas the two restorer lines of released hybrids used as susceptible pollen parents had 26% and 88% ergot severity (Table 1). It is interesting to note that H 77/833-2, the restorer parent of commercial hybrids (HHB 60, HHB 67, and HHB 68), which

was not specifically bred for ergot resistance, had 26% ergot severity that was comparable to that of a resistance source ICMER 127 (17% ergot severity).

#### *Selfed seedset in parental lines*

All the A-lines had no selfed seedset (Table 1). Among B-lines, 843B had 32% selfed seedset and others had 59–75% selfed seedset. Selfed seedset of pollen parents ranged from 46 to 79%.

Table 2. Mean squares for ergot severity (%) and selfed seedset (%) of pearl millet hybrids, 1991 and 1992 rainy seasons

Source of variation	df	Mean square	
		Ergot severity	Seedset
Year	1	1576	195
Replication/year	4	245	7
Hybrid	55	5930**	77192**
Seed parent (SP)	7	24371**	50522**
Cytoplasm (C)	1	57095**	351583**
Nuclear genetic resistance (R)	1	102747**	642
C × R	1	31	817
Pollen parent (PP)	6	17837**	3975**
SP × PP	42	1155**	1120**
C × PP	6	1924**	5601**
R × PP	6	3861**	391**
(C × R) × PP	6	1115**	604**
Year × Hybrid	55	143*	144
Year × SP	7	150	115
Year × C	1	76	43
Year × R	1	711	181
Year × (C × R)	1	107	17
Year × PP	6	72	158
Year × SP × PP	42 (41) <sup>1</sup>	152*	147
Residual	219 (208) <sup>1</sup>	100	132

<sup>1</sup> Degrees of freedom for seedset.

\* Significant at  $P = 0.05$ .

\*\* Significant at  $P = 0.01$ .

### Ergot severity in hybrids

Effects of seed parents, pollen parents and seed parent × pollen parent interactions contributing to variation for ergot severity among hybrids were all highly significant ( $P < 0.01$ ) whereas interactions of both seed parents and pollen parents with years were not significant (Table 2). The variation attributable to seed parents had large components due to cytoplasmic and nuclear genetic resistance, both of which were highly significant, but the effect of nuclear genetic resistance was about 1.8 times larger than those due to cytoplasm. Similarly, the effect of resistance × pollen parent interactions was about twice as large as that of cytoplasm × pollen parent interaction.

Hybrids developed by crossing all seed parents with the most susceptible pollen parent (ICMR 356) had high ergot severity, ranging from 72 to 96% (Table 3). When this pollen parent was involved, there were no significant differences in the ergot severities of hybrids with A-lines and those with the correspond-

ing B-lines, and between hybrids of susceptible seed parents and those on resistant seed parents. Even where a relatively less susceptible pollen parent (H 77/833-2) was involved, ergot severities of hybrids of susceptible A-lines were not significantly different from those of hybrids of the corresponding B-lines. However, hybrids of this pollen parent made on the two resistant A-lines (ICMA 91113 and ICMA 91115) were significantly more susceptible (87% and 54% ergot severity) than the hybrids made on their counterpart B-lines (56% and 38% ergot severity).

Among hybrids of the two susceptible A-lines, those with resistant pollen parents were as susceptible as those with susceptible pollen parents, for all practical purposes. Among hybrids involving resistant pollen parents, those made on all A-lines were significantly more susceptible than those made on their corresponding B-lines. There were, however, two notable exceptions: though the hybrids of a highly resistant A-line (ICMA 91115) made with two highly resistant pollen parents (ICMER 66 and ICMER 92) were slight-

Table 3. Ergot severity (%) of pearl millet hybrids based on ergot susceptible and resistant parental lines. Mean of 1991 and 1992 rainy seasons

Seed parent	Ergot severity in hybrids with pollen parents						
	Susceptible		Resistant				
	ICMR 356	H 77/833-2	ICMER 97	ICMER 127	ICMER 66	ICMER 92	ICMER 98
Susceptible							
81A	90	90	82	84	80	81	67
81B	91	81	56	51	44	37	45
843A	94	94	94	82	89	80	88
843B	87	81	61	46	53	67	38
Resistant							
ICMA 91113	96	87	86	75	27	25	31
ICMB 91113	82	56	18	22	6	1	11
ICMA 91115	84	54	72	38	11	8	19
ICMB 91115	72	38	18	8	3	3	3
LSD (0.05 = 13.8							

ly more susceptible (8–11% severity) than the corresponding hybrids of ICMB 91115 with the same two pollen parents (3% severity), the differences were not statistically significant (Table 3). There were significant positive correlations ( $r = 0.80$  and  $0.81$ ) ( $P < 0.05$ ) between the ergot severity of pollen parents and that of their hybrids with each of the resistant A-lines.

#### *Selfed seedset in hybrids*

There were highly significant effects ( $P < 0.01$ ) of seed parents, pollen parents, and seed parent  $\times$  pollen parent interaction on seedset of hybrids (Table 2). Almost all of the seed parent effect was accounted for by the cytoplasm effect. The cytoplasm  $\times$  pollen parent, nuclear genetic resistance  $\times$  pollen parent, and nuclear genetic resistance  $\times$  cytoplasm  $\times$  pollen parent interactions were also highly significant, but cytoplasm  $\times$  pollen parent interaction was the largest component. There was no significant variation among B-line hybrids of either ICMR 356 (86–93% selfed seedset) or H 77/833-2 (78–87% selfed seedset). However, the variation among A-line hybrids with either of these two pollinators was significant and these hybrids had significantly less selfed seedset than the hybrids of their corresponding B-lines (Table 4).

B-line hybrids developed with resistant pollen parents had 73–92% selfed seedset, similar to that of B-line hybrids developed with susceptible restorer lines (Table 4). Hybrids of the corresponding A-lines had significantly less selfed seedset: 29–71% when sus-

ceptible restorer lines were involved and 0–43% when resistant pollen parents were involved.

#### *Protogyny*

The relatively less resistant A-line ICMA 91113 (26% ergot severity) had a protogyny of 105 h, which was significantly longer than the 74 h protogyny of the highly resistant A-line ICMA 91115 (8% ergot severity) (Table 5). In both ergot resistant A/B pairs, A-lines had significantly longer protogyny than their corresponding B-lines. In general, there was no significant difference among either the hybrids of an A-line or among the hybrids of a B-line. Hybrids of A-lines, however, had significantly longer protogyny (73–90 h) than those of the corresponding B-lines (43–62 h).

#### **Discussion**

All A-lines with no selfed seedset (32–75% selfed seedset in B-lines) produced hybrids that had significantly less seedset than hybrids of their corresponding B-lines. This reduced selfed seedset, however, was not always accompanied by significantly higher ergot susceptibility, neither for the A/B lines nor for their hybrids. Two distinctly divergent situations emerged where there were no significant differences in ergot severity between A-lines and B-lines as well as between hybrids of A-lines and those of B-lines. In the first, B-lines (and hence A-lines) were highly

Table 4. Selfed seedset (%) in pearl millet hybrids based on ergot-susceptible and resistant parental lines. Mean of 1991 and 1992 rainy seasons

Seed parent	Selfed seedset in hybrids with pollen parents						
	Susceptible		Resistant				
	ICMR 356	H 77/833-2	ICMER 97	ICMER 127	ICMER 66	ICMER 92	ICMER 98
Susceptible							
81A	29	44	1	0	25	21	25
81B	93	78	85	83	89	91	89
843A	55	71	8	6	16	44	16
843B	86	87	92	92	86	73	86
Resistant							
ICMA 91113	46	55	1	0	0	31	0
ICMB 91113	87	86	88	92	87	81	90
ICMA 91115	48	40	20	0	0	23	0
ICMB 91115	89	82	87	91	90	74	92
LSD (0.05) = 13.9							

Table 5. Protogyny length of ergot-resistant parental lines and their hybrids, 1992 rainy season

Seed parent	Protogyny length (h)			
	Seed parents	Hybrids of pollen parents		
		ICMER 92	ICMER 97	ICMER 98
ICMA 91113	105	73	87	90
ICMB 91113	64	62	44	51
ICMA 91115	74	73	81	82
ICMB 91115	52	56	46	43
SE		± 4.9		

susceptible and hybrids of these A/B pairs involving susceptible pollen parents were all susceptible. In the second, a highly resistant A/B pair was involved and their hybrids with highly resistant pollinators were all resistant.

When a resistant A-line had a moderate disease severity level (ca. 25%), the counterpart B-line had a significantly lower ergot severity. In hybrids from susceptible × resistant crosses, and those from resistant × resistant crosses where one or both parents had moderate resistance levels, A-line hybrids were significantly more susceptible than the corresponding B-line hybrids. In these two groups of hybrids, there were highly significant negative correlations between selfed seedset and ergot severity ( $r = -0.91$  when susceptible A/B pairs were involved and  $r = -0.61$  when resistant A/B pairs were involved).

Ergot resistant A-lines and their hybrids with resistant pollen parents had significantly longer protogyny than the corresponding ergot resistant B-lines and their hybrids. These results are similar to those reported earlier (Thakur et al., 1991). However, this longer protogyny was not always associated with significant increases in ergot severity. Thus, longer protogyny accounted only for a part of the higher ergot severities of A-lines and their hybrids, as did selfed seedset, a measure of male fertility. Nuclear genetic resistance to ergot more than compensates for the effects of this cytoplasm in increasing the susceptibility of A-lines and their hybrids. Thus, by breeding highly resistant A-lines and pollen parents, it is possible to develop highly resistant hybrids (having about 10% ergot severity under artificially inoculated conditions) even if their

male fertility is poor to moderate and protogyny is longer.

A-line hybrids registering 25–30% ergot severity under artificial inoculation could be produced on even a moderately resistant A-line, if pollen parents were highly resistant (< 5% ergot severity). In the ergot-endemic areas such hybrids might be a viable proposition for genetic management of ergot as ergot severities of that magnitude under artificial inoculation have been shown to provide adequate levels of functional field resistance (Thakur et al., 1989a). The commercial viability of these hybrids, however, will depend on their yield potential in comparison to open-pollinated varieties and the extent of ergot susceptibility of the latter under natural ergot pressure in those areas. In this context, the present study also showed that in case of moderately resistant A-lines, use of the counterpart B-lines as seed parents in crosses with even moderately resistant pollen parents produced hybrids having 18–22% ergot severity. These hybrids can also be expected to provide adequate levels of functional field resistance under natural epidemic conditions.

It must be recognized that the use of male-fertile inbred lines as seed parents would lead to impure hybrid seed stocks that would contain a small proportion of seed of the seed parental line. The economic viability of such variable hybrids would need to be assessed in terms of their acceptance by farmers and hybrid seed industries, especially in areas where phenotypic uniformity of the cultivars is a major requirement. Such hybrids, however, will have less severe ergot due to their higher male fertility, shorter protogyny, and better seedset. It has also been shown (Kiula et al., 1991) that yield potential of these hybrids is not significantly different from those of pure single-cross hybrids.

### Acknowledgement

We sincerely thank Dr. C.T. Hash for his several valuable comments on an early version of this manuscript.

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