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Flowering events in relation to smut susceptibility in pearl millet

R. P. THAKUR
Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

In field experiments at ICRISAT, selected lines of pearl millet (*Pennisetum glaucum*) resistant and susceptible to smut (*Tolyposporium penicillariae*) were evaluated for the timing of flowering events. In smut-resistant lines, the time from the boot-leaf stage (inoculation) to stigma emergence varied in the range 46–120 h, from boot to anther emergence 105–190 h, and from stigma emergence to anther emergence (protogyny period) 59–131 h; in smut-susceptible lines, the corresponding periods were 62–140 h, 146–200 h and 44–120 h, respectively. There were no significant correlations between timing of events and smut severity. Three cytoplasmically male-sterile lines showed longer protogyny periods and higher smut severity than their corresponding maintainer lines. Four lines having short protogyny periods (22–52 h) and resistance to ergot (*Claviceps fusiformis*) also showed high resistance to smut. Resistance to smut in most ergot-susceptible lines was independent of the timing of flowering events, but in ergot-resistant lines it could be closely related to flowering events.

INTRODUCTION

Flowering in pearl millet (*Pennisetum glaucum*) is protogynous, i.e. the stigmas emerge and mature before the stamens. This characteristic of the species makes it highly cross-pollinated, but predisposes it to infecton by ovary-infecting fungal pathogens. *Tolyposporium penicillariae*, the causal agent of smut, infects the ovary through the young emerging stigma (Bhatt, 1946). Pollination of panicles inoculated with *T. penicillariae* has been shown to reduce smut infection (Thakur *et al.*, 1983a; Wells *et al.*, 1987), and it is generally believed that cross-pollination prevents infection under natural conditions. In a field-based smut screening technique (Thakur *et al.*, 1983b), cross-pollination is prevented by covering the panicles with parchment bags immediately after inoculation at the boot-leaf stage, and removing the bags 20 days after inoculation when development of smut sori and seeds are complete. This technique has been used successfully to identify a number of lines highly resistant to smut at ICRISAT and other locations (Thakur *et al.*, 1986). The bagging of inoculated panicles, however, does not eliminate self-pollination which may be involved in reduced smut infection.

In ergot disease of pearl millet, caused by *Claviceps fusiformis*, early pollination of inoculated panicles has been shown to reduce infection (Thakur & Williams, 1980), and the screening technique designed to prevent cross-pollination (Thakur *et al.*, 1982) has been used successfully in identifying sources of resistance. This resistance is apparently based on short protogyny, which results in rapid self-pollination in the inoculated, bagged panicles. In pearl millet panicles, stigma withering initiates within 3 h, and complete withering occurs within 6 h after pollination (Thakur & Williams, 1980; Willingale *et al.*, 1986). The withering of stigmas is caused by the development of a localized constriction in the basal tissue of the style within 6 h of pollination, thus isolating the ovary from the stigma and preventing subsequent infection by *C. fusiformis* (Willingale *et al.*, 1986). The mechanism of resistance to *T. penicillariae* is, however, not clear. In resistance screening at ICRISAT, all ergot-resistant lines (having short protogyny) showed high levels of resistance to smut (Thakur *et al.*, 1985; Thakur & King, 1988a), but none of the smut-resistant lines showed resistance to ergot (R. P. Thakur, unpublished). This study was

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planned to determine the relationship between the time course of flowering events and susceptibility to smut in selected lines of pearl millet.

MATERIALS AND METHODS

Field experiments were conducted during the 1985 dry and rainy seasons, and the 1987 and 1988 rainy seasons at ICRISAT. During the 1985 dry and rainy seasons eight pearl millet lines, five resistant to smut (< 5% mean severity) and three susceptible (> 5% mean severity) were grown, each in a two-row plot, 4 m long with 20 plants per row. Observations on flowering events were taken during the dry season, and on smut and ergot severities during the rainy season. In the 1987 rainy season, six pearl millet lines, four resistant to ergot (< 5% mean severity) and two susceptible (> 5% mean severity) were grown, each in a two-row plot, 4 m long with three replications in a randomized block design, for observations on protogyny and reactions to ergot and smut. During the 1988 rainy season 12 pearl millet lines, eight smut susceptible—including three cytoplasmically male-sterile (A) lines with their corresponding maintainer (B) lines—and four smut-resistant inbreds were grown, each in a two-row plot, 4 m long with two replications in a randomized block design, for observations on flowering events, smut reaction and seed set.

Observations of flowering events

In each plot, panicles of 10 plants in the 1985 dry season and the 1988 rainy season and 20 plants in the 1987 rainy season were covered with parchment pollination bags at the boot-leaf stage (boot) with date and time of bagging noted on the bag, and also on the data record sheet. On each panicle, by briefly opening the bag daily at 09.00 and 16.00 h, the time from the boot to initiation of stigma emergence, and to anther emergence was assessed.

Smut inoculation

In each plot the main shoot panicles of 20 plants in the 1985 and 1987 rainy season and 10 in the 1988 rainy season were inoculated by injecting a suspension of *T. penicillariae* sporidia (10^6 sporidia per ml) into the boot leaf sheath, and covering the panicles with parchment bags (Thakur *et al.*, 1983b). High humidity, essential for infection and disease development, was created by operating an overhead sprinkler for 30 min between 12.00 13.00 and 17.00–18.00 h, on dry days. Twenty days after inoculation, bags were removed and panicles were scored for smut infection by reference to a smut severity rating scale (Thakur & King, 1988b). In the 1988 rainy season experiment, observations on flowering events and seed set were recorded in each plot on the same panicles used for smut inoculation, while in other experiments different plants were used.

Ergot inoculation

In each plot, plants were inoculated at the full stigma emergence stage with a suspension containing 10^6 conidia per ml of *C. fusiformis* (10 plants in the 1985 rainy season and 20 plants in the 1987 rainy season). Panicles were protected from cross-pollination by covering them with parchment bags (Thakur *et al.*, 1982). High humidity was maintained as for smut inoculation. Bags were removed 15 days after inoculation and ergot was assessed with the aid of a severity rating scale (Thakur & Williams, 1980).

RESULTS

Time from boot to stigma emergence and smut severity

The time from inoculation at the boot stage to the estimated time for initiation of infection (stigma emergence) varied among the lines. In smut-resistant lines it varied from 46 to 91 h during the dry season (Table 1) and from 65 to 120 h in the rainy season (Table 2), and in smut-susceptible lines this period varied from 62 to 84 h in the dry season and 63 to 140 h in the rainy season. The smut-resistant lines ICMPS 100-5-1 and ICMPS 900-9-3, common to the two tests, recorded boot to stigma emergence periods of 64 and 89 h in the dry season, and 65 and 79 h, respectively, in the rainy season. Although there were significant differences between lines, the resistant and susceptible lines could not be discerned on this basis, and there was no significant correlation between the mean value for boot to stigma emergence time and the mean smut severity.

Time from boot to anther emergence and smut severity

In smut-resistant lines the time from boot to anther emergence varied from 105 to 190 h, and in smut-susceptible lines from 174 to 186 h during the dry season (Table 1) and from 132 to 189 h in

Table 1. Average period of flowering events, smut severity and ergot severity in selected pearl millet lines, ICRISAT, 1985

Line	Time (h)[a]		Protogyny period (h)[a]	Smut severity (%)[b]	Ergot severity (%)[c]
	Boot-SE	Boot-AE			
Smut resistant					
ICMPS 100-5-1	64	151	87	0	43
ICMPS 100-5-3-3	59	190	131	0	75
ICMPS 900-5-4-14	46	105	59	0	86
ICMPS 900-9-3	89	168	79	0	60
ICMPS 1600-2-9-1	91	190	99	0	79
Smut susceptible					
BJ 104	62	174	111	90	94
5141A	84	186	102	91	92
ICH 206	78	179	100	85	90
SE	5·7	10·1	7·6	16·2	6·4

[a] Based on mean of 10 panicles during the 1985 season.
[b] Based on mean of 20 inoculated panicles in the 1985 rainy season smut nursery.
[c] Based on mean of 10 inoculated panicles in the 1985 rainy season ergot nursery.
SE, stigma emergence; AE, anther emergence; protogyny period = time between SE and AE.

Table 2. Average period of flowering events, smut severity and seed set in selected pearl millet lines, ICRISAT, rainy season 1988

Line	Time (h)[a]		Protogyny period (h)[a]	Smut severity (%)[a]	Seed set (%)[a]
	Boot-SE	Boot-AE			
Smut resistant					
SRB 3	76	132	60	4	86
ICMPS 900-9-3	79	170	91	0	73
ICMPS 100-5-1	65	143	78	0	79
ICMSR 11	120	189	71	0	84
Smut susceptible					
841A	86	159	74	90	< 1
841B	79	146	67	47	39
81A	82	200	118	88	< 1
81B	90	190	101	70	7
842A	63	183	120	63	0
842B	66	155	90	13	6
SRB 1	80	170	90	10	73
SRB 2	140	183	44	12	63
SE	5·2	7·4	3·9	2·8	2·9

[a] Mean of 10 panicles in each of two replications; the same panicles were used for recording flowering events and smut inoculations.
SE, stigma emergence; AE, anther emergence; protogyny period = time between SE and AE.

smut-resistant lines and 146 to 200 h in smut-susceptible lines in the rainy season (Table 2). The smut-resistant lines ICMPS 100-5-1 and ICMPS 900-9-3 recorded mean times of 151 and 168 h in the dry season, and 143 and 170 h in the rainy season, respectively. Although there were significant differences among lines for this trait, there was no significant correlation of the mean period between boot and anther emergence with the mean smut severity.

Protogyny and smut severity

The mean protogyny period varied from 59 to 131 h in the dry season and 71 to 91 h in the rainy season in smut-resistant lines, and from 100 to 111 h in the dry season and 44 to 120 h in the rainy season in smut-susceptible lines (Tables 1 and 2). There were significant differences among lines for this trait, but there was no significant correlation between the mean protogyny period and the mean smut severity levels.

Two of the A lines, 81A and 842A, exhibited significantly longer protogyny periods (74–120 h) and higher smut severities (63–90%) than their corresponding B lines, which had the protogyny periods of 67–101 h and smut severities of 13–70% (Table 2). Among the three A/B pairs, 842A/B was least susceptible, although it did not show the shortest mean protogyny period.

Ergot severity

Ergot severity in smut-resistant or smut-susceptible lines with protogyny periods of 59–131 h varied from 43 to 92% (Table 1). Four ergot-resistant lines (< 5% mean severity) with mean protogyny periods of 22–52 h all showed complete resistance to smut, while the two ergot-susceptible lines (> 5% mean severity) BJ 104 and BK 560, with 91 and 94 h mean protogyny periods, respectively, showed high susceptibility (80–85% severity) to smut (Table 3).

Seed set

Seed set was generally higher in smut-resistant lines (79–86%) than in smut-susceptible lines (7–73%) (Table 2). Two moderately smut-susceptible lines, SRB 1 (10% severity) and SRB 2 (12% severity), had 73 and 63% seed set, respectively. There was, however, no significant correlation between the mean protogyny period and the mean seed set.

DISCUSSION

A generalized time course of flowering events with the smut inoculation and symptom development timings superimposed is shown in Fig. 1. From the time of inoculation at the boot-leaf stage, it takes 3–5 days for *T. penicillariae* sporidia to infect the young stigmas emerging from the glumes of the florets (Bhatt, 1946). During this period, any change in the environment that is unfavourable for sporidial survival might lead to reduced infection. Infection is probably completed in the period from the boot stage to anther emergence and most stigmas would wither in bagged, inoculated panicles; stigmas that remain fresh could receive pollen to facilitate fertilization and thereby prevent smut infection (Thakur *et al.*, 1983a; Wells *et al.*, 1987). In pearl millet, the time course of flowering events could vary particularly with temperature, relative humidity, and the plant phenotype. In the normal growing season a cultivar takes 15–21 days from the boot-leaf stage to the visible grain filling stage, while smut sori appear within 10–14 days of inoculation at boot. Of the three measured intervals between flowering events, boot to stigma emergence, boot to anther emergence, and stigma emergence to anther emergence (protogyny), protogyny seems to be the most important in the cultivars for which smut resistance is related to pollination.

The results indicated that resistance to smut in ergot-resistant cultivars is probably associated with self-pollination and fertilization, but in other cultivars it is not associated with self-pollination. Under natural conditions, escape from smut infection can be attributed to cross-pollination (Thakur *et al.*, 1983a), as with ergot (Thakur & Williams, 1980; Thakur *et al.*, 1983d).

Thakur *et al.* (1983a) and Wells *et al.* (1987) demonstrated that smut-inoculated panicles when pollinated at full-stigma emergence, i.e. 3–4 days after inoculation, showed significantly less smut infection. This indicates that smut infection can be enhanced in the absence of pollen, and suggests that lines with a longer protogyny period would be more susceptible. This hypothesis is supported by the fact that ergot-resistant lines, which generally have shorter protogyny than ergot-susceptible lines, have shown high levels of smut resistance. Conversely, all the smut-resistant lines that do not have short protogyny are highly susceptible to ergot (R. P. Thakur, unpublished). In this study, smut-resistant lines with 59–132 h protogyny recorded 43–86% ergot severity

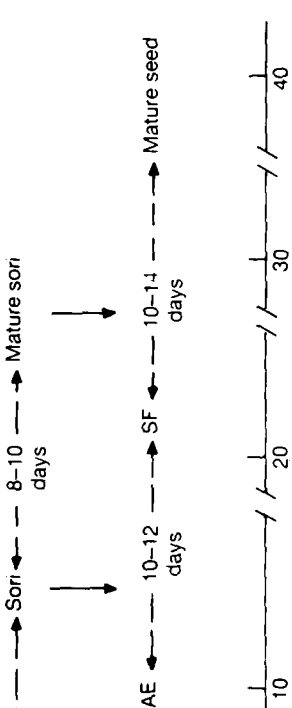

Fig. 1. A generalized scheme for the time course of flowering events in pearl millet, with smut inoculation, infection, and sorus development stages superimposed. Inoc., inoculation of panicle at the boot-leaf stage; Inf, likely initiation of infection through stigma (Bhatt, 1946); Sori, appearance of smut sori in florets; Boot, the boot-leaf stage; SE, initiation of stigma emergence from the florets; AE, initiation of anther emergence and pollen shedding; SF, appearance of seed formation.

Table 3. Protogyny period, smut severity and ergot severity of pearl millet lines resistant and susceptible to ergot, ICRISAT, rainy season 1987

Line	Protogyny period (h)[a]	Ergot severity (%)[a]	Smut severity (%)[a]
Ergot resistant			
ICMPES 1	22	< 1	0
ICMPES 2	52	< 1	0
ICMPES 29	43	< 1	0
ICMPES 34	37	4	0
Ergot susceptible			
BJ 104	94	92	80
BK 560	91	94	85
SE	12·0	10·8	17·4

[a] Based on mean of 20 panicles in each of three replications.

(Table 1), while ergot-resistant lines with 22–52 h protogyny showed complete resistance to smut (Table 3). From this, it seems that lines with a protogyny period of more than 52 h are likely to be susceptible to ergot, but not to smut because protogyny periods in smut-resistant lines were 59–131 h. In 1985, timings of flowering events were recorded in the dry season, and smut and ergot scorings were taken in the rainy season, but the results of 1985 are in agreement with the 1988 rainy season data for the two common lines, ICMPS 100-5-1 and ICMPS 900-9-3, indicating insignificant effect of seasons on the timings of flowering events.

The longer protogyny and higher smut severity of A lines could be associated with the cytoplasmic male-sterility system; in B lines, production of pollen and partial self-pollination under bagged panicles might account for reduced smut infection. High smut susceptibility of most of the F_1 hybrids could be attributed to their male-sterile cytoplasm. In a study with five A lines and their corresponding B lines, and hybrids made on them, Khairwal *et al.* (1986) reported no significant difference for smut susceptibility between hydrids made with A and B lines, and the lower smut susceptibility of B lines than of A lines was attributed to rapid pollination in B lines. They concluded that male-sterile cytoplasm was not related to smut susceptibility of the hybrids. However, they did not provide data on protogyny and seed set of the parental lines and the hybrids. In other lines the period of protogyny was not correlated with smut severity levels. It could be argued that lines with short protogyny would have good seed set under selfing and would have resistance to smut. Smut resistance, however, is not associated with good seed set; a number of highly smut-resistant lines with very few or no seed set under selfing were observed. It seems that the amount of seed set depends on several factors that are not related to smut resistance.

Smut epidemics in pearl millet are unlikely because the relatively long latent period (about 20 days) drastically reduces the chance of secondary infection in a crop, and the occurrence under natural conditions of cross-pollination reduces smut infection. This hypothesis is well supported by the fact that so far there has not been even a single report of an epidemic, despite the survival of the pathogen in most areas of the world where pearl millet is grown (Rachie & Majmudar, 1980; Thakur & King, 1988b). Smut in severe form does occur, however, when cross-pollination is reduced during the rainy period at flowering. Then the F_1 hybrids with their more synchronous tillering and flowering generally develop more smut than the heterogeneous open-pollinated varieties.

Because of increasing commercial cultivation of F_1 hybrids in India, resistance to smut has become an important component of the hybrid breeding programme at ICRISAT. A number of pearl millet lines that have high levels of resistance to smut (but not to ergot) and desirable agronomic traits have been identified (Thakur & Chahal, 1987; Thakur & King, 1988b), and several of these are being utilized in the programme. Recent studies on inheritance of smut resistance have indicated that resistance is controlled by a few dominant genes with additive effects (Chavan *et al.*, 1988). Utilization of smut resistance based on short protogyny might be difficult to transfer as the inheritance of resistance could be complex, like that reported for ergot (Thakur *et al.*, 1983c).

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