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An *in vitro* screening technique for the identification of grain mould resistance in sorghum

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ABSTRACT: Grain mould (GM) is the most important biotic constraint of sorghum *[Sorghum bicolor* (L.) Moench] in India and other parts of the world and host plant resistance is the most convenient method of control of this disease. Breeding for disease resistance has been slow due to the total reliance on field screening which is used once-a-year during rainy season. In order to conduct screening independent of season and to detect possible escapes from field screening, an *in vitro* screening technique has been developed. The technique involves dip inoculation of mature seed in a mixed spore suspension (1×10^6 spores ml^{-1}) of major GM fungi *(Fusarium moniliforme, F. pallidoroseum, and Curvularia lunata),* transferring inoculated seed to the pre-sterilized petridish humid chamber @ 25 seed $plate^{-1}$, and their incubation at 28±1°C with or without light for 5 days. Visual evaluation of mould infection was done on 1-9 scale (1 = no colonization, and 9 = >75% seed surface colonization by the mould fungi) using an illuminated magnifier. The efficiency of the technique was measured by comparing mean grain mould scores (MGMS) of 43 accessions evaluated at Bhavanisagar, Mysore and Patancheru in India. There was a significant correlation ($P<0.01$) between *in vitro* MGMS and field scores at or across three locations. Of the 66 photoperiod sensitive accessions that were screened using the *in vitro* screening, 14 showed high level of resistance even under field disease nursery situation. The usefulness of the technique in GM resistance program is discussed.

Key words: Grain mould, *Fusarium* sp., *Curvularia* sp., screening technique

Grain mould is a major biotic, yield reducing constraint in early maturing, sorghum cultivars worldwide where they flower and fill grains during rainy days. Cultivars with white grains are particularly vulnerable. Grain yield losses from 30 to 100% depending upon cultivar, time of flowering, and maturity, and soil types have been reported (Williams and Rao, 1980; S.D. Singh and S.S. Navi, unpublished). The disease drastically reduces grain quality and impair seed germination. Several mycotoxins including zearalenone, Tenuazonic acid, etc., have been found associated with moulded grains (Forbes *et al.,* 1992). Consumption of moulded grain has caused headache, fever, general body heat and eye burning.

The use of mould resistant cultivars is the most convenient method of control of this disease. One of the prerequisites to the development of resistant cultivars is the availability of a reliable screening technique (Bandyopadhyay and Mughogho 1988). Although, a field screening technique is available, it has two limitations: (i) it can be used only in rainy season, thereby reducing the pace of resistance breeding and (ii) it does not allow screening of photoperiod sensitive material constituting 75% of the total world germplasm. In this paper, we describe an *in vitro* screening technique and its use in the identification of resistance to grain mould even in photoperiod sensitive accessions.

MATERIALS AND METHODS

Isolation and multiplication of grain mould fungi

Moulded grain collected from 1992 rainy season were surface sterilized in 0.1% $HgCl_2$ for 2 min, washed with several changes in distilled sterile water and transferred aseptically on oat meal agar. The plates were incubated at 28±1°C under 12 h light cycle for 10 days. Most commonly occurring fungi were *Fusarium moniliforme, F. pallidoroseum* and *Curvularia lunata.* These three fungi were selected for developing an *in vitro* screening technique. The conidia of these fungi were produced on grains of a sorghum cultivar SPV 104 placed in 150 ml conical flasks after soaking in sterile distilled water (SDW) for 4 h followed by sterilizing the flasks at 121°C and 15 lb psi for 15 min. The grains kept in such flasks were separately inoculated with the three fungi and incubated at 28±1°C

under 12 h light cycle in an incubator for 7 days. During this period the flasks were shaken every alternate day for the development of uniform growth of fungi on all the grains.

Inoculation procedure

Twenty grains infected with individual fungus were aseptically transferred into a 50 ml beaker containing 15 ml SDW. The beakers were shaken on Vortex mixer and the suspension decanted through a tea strainer. The spore concentration in the resultant suspension was adjusted to 1×10^6 spores ml^{-1} using a haemocytometer. A mixed spore suspension of the three fungi was prepared by taking equal volume of spore suspension of each fungus and used in all the tests.

Seeds of a sorghum test line were immersed for 1-2 min in a petridish (5 cm) containing 10 ml mixed spore suspension of the three fungi prepared as above. Then twenty five inoculated seeds were aseptically transferred to a pre-sterilized petridish humidity chambers. The humidity chamber was prepared by lining the lower lid with a layer of absorbent cotton followed by two layers of blotting paper. The cotton-blotting paper were wetted with 15 ml water. Upon covering upper lid, the entire assembly was placed in petridish sterilizing container and sterilized at 121°C and 15 PSI for 15 min. The humidity chambers, containing inoculated seeds, were incubated at 28±1°C under 12 h light cycle for five days and mould severity was recorded on a 1-9 rating scale (1 = no mould, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%. 5 = 21-30%, 6 = 31-40%, 7 = 41-50%, 8 = 51-75%, 9 = >75% grain surface areas covered by the mould).

Sixty-six photoperiod sensitive accessions belonging to eight sorghum races collected from seven countries namely, Burkina Faso, Botswana, Malawi, Sierra Leone, India, Tanzania, and Nigeria were evaluated (Table 1).

Five-day-old seedlings of all the accessions that developed low mould severity (1-3 scores) in *in vitro* screening were transplanted in 25-cm-dia. plastic pots containing soil-fertilizer mixture. Twenty-day-old seedlings were exposed to reduced day length of 8 h day^{-1} for four weeks by covering the pots with a black Polythene sheet. At boot leaf stage, the pots were transferred to field, where overhead sprinkler remained in operation after panicle emergence till 15 days after physiological maturity. Then, the mould severity was recorded in each genotype on 1-9 scale.

Refinement of Screening Procedure

Five experiments were conducted to determine the variation effect of different components of the inoculation procedure on the development of mould severity using three fungi (as mentioned above) and two germplasms — IS 9471 (resistant) and Bulk Y (susceptible).

For component (i) effect of pre-inoculation seed soaking duration: Seeds of each cultivar were soaked in distilled sterile water — 4, 6, 8, 16 and 24 h period separately. The excess water after soaking the seeds was decanted and seeds were dried using paper towel. Each seed lot was then dip-inoculated with the mixed spore suspension using the method described earlier. For component (ii) effect of spore concentration: seeds of these germplasm were dip-inoculated in five concentrations - 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores ml^{-1} of mixed spore suspension. Whereas, for component (iii) effect of incubation period: Seeds of each germplasm were inoculated with a concentration of 1×10^6 spores ml^{-1} and incubated for 24, 48, 72, 96,

Table 1. *In vitro* evaluation of 66 sorghum accessions for grain mould[1] resistance

Sorghum (Race/subrace)	Number of accessions	Mean mould score[2]				
		1	3	5	7	9
Conspicuum	10	0	1	7	2	0
Durra	5	0	2	2	1	0
Durra-Bicolor	10	0	3	6	1	0
Durra-Caudatum	1	0	0	1	0	0
Guineense	10	0	1	3	4	2
Guinea-Caudatum	1	0	1	0	0	0
Rox burghii	19	0	8	5	3	3
S. margaritiferum	10	0	1	4	2	3
IS 9471 (Resistant check)		0	0	1	0	0
Bulk-Y (Susceptible check)		0	0	0	0	1

[1] Using three major grain mould fungi — *Fusarium moniliforme, F. pallidoroseum and Curvularia lunata*

[2] Evaluated on a 1-9 rating scale, where 1 = free from colonization and 9 = >75% colonization

and 120 h duration. In case of component (iv), effect of temperature: Seed of the germplasm were inoculated with a concentration of 1×10^6 spores ml^{-1} and were incubated for 120 h in a thermogradient temperature plate at 14, 21, 28, 35 and 42°C. Whereas, for component (v) effect of light and darkness: Seeds inoculated with a concentration of 1×10^6 spores ml^{-1} were incubated under three light regimes, 12 h light cycle, 24 h light cycle, and 24 h dark cycle at 28±1°C for 120 h.

RESULTS AND DISCUSSION

Sixty six photoperiod sensitive accessions belonging to eight sorghum races that were screened using the *in vitro* screening procedure showed conspicuous differences in their mould reaction (Table 1). No accession was noted mould free. However, 17 accessions belonging to races conspicuum, guineense, guinea-caudatum and margaritiferum (one each), durra (2), durra-bicolor (3), and rox burghii (8) showed a rating of 3 and 41 other accessions had mould ratings ranging from 5-7. The remaining accessions were highly susceptible. The resistant check, IS 9471 was rated 3 and susceptible, Bulk Y rated 9 on 1-9 scale. The mould reaction of potted plants of 14 accessions (3 of the 17 accessions did not grow) exhibited highly resistant reaction (rating 2) under *in vitro* test. However, only four accessions (IS 7173. IS 23773, IS 23783 and IS 34219) were found free from mould infection and others exhibited high level of mould resistance (<3 rating) grown under field condition in 1992 rainy season. Reaction of these accessions are presented in Table 2.

Both R and S sorghum germplasms reacted almost similarly to most of the component factors used to determine mould severity (Fig. 1a-e). Up to 8 h of pre-soaking of seed in water prior to inoculation did not affect the mould reaction. However, a decrease in mould severity was seen in case where pre-soaking duration was for more than 8 h. Increase in spore concentration from 1×10^4 to 1×10^8 did not increase the mould severity in resistant genotype, IS 9471. However, in the susceptible cultivar, Bulk Y, spore concentration has shown significant differences in mould severity. However, the differences were not significant (Fig. 1b). Highest mould severity developed in both the germplasms when inoculated grains were incubated at 28°C, followed by at 35°C (Fig 1c). There was gradual increase in mould severity in both the germplasms with increase in incubation period after the inoculation and the highest score of 8.5 was recorded after 5 days of incubation (Fig. 1d). Whereas, the light and/or dark appear to have no effect on the development of mould in both the cultivars (Fig. 1e).

Table 2. Mould reactions of 14 photoperiod sensitive sorghum accessions *in vitro* and in field disease nursery

Sorghum (race/ subrace)	Accessions	Mean grain mould score[a]	
		in-vitro	Field disease nursery
Conspicuum	IS 7173	2	1
Conspicuum	IS 23773	3	1
Conspicuum	IS 23783	3	1
Conspicuum	IS 24173	3	2
Guineense	IS 7326	2	3
Guineense	IS 2157	3	2
Rox-burghii	IS 4963	3	2
Rox-burghii	IS 5726	2	2
Rox-burghii	IS 4011	2	2
Rox-burghii	IS 5292	2	2
Rox-burghii	IS 5727	4	2
Rox-burghii	IS 34219	2	1
Margaritiferum	IS 27761	3	2
Margaritiferum	IS 27681	3	2
Control (Res.)	IS 9471	3	2
Control (Susc.)	Bulk-Y	9	9

[a] mould score on 1-9 scale, where 1=free from colonization and 9 = >75% colonization

A range of mould resistance expressed by various photoperiod sensitive accessions indicated that the technique can differentiate resistant and susceptible Genotypes. High level of resistance shown by the resistant genotype, IS 9471 (Bandyopadhyay *et al.* 1988) further confirms its reliability in identifying resistance that is effective under field conditions. In a comparative study, a set of 45 sorghum lines were tested for mould reaction using the refined inoculation technique described here and under natural field screening at three locations in India, namely, Bhavanisagar, Mysore, and Patancheru during 1995. The mean grain mould score (MGMS) of the lines from *in vitro* tests were highly correlated with MGMS obtained from three locations' data at 1% level of significance (S.D. Singh and S.S. Navi, unpublished). The technique is not only reliable, but also economical, repeatable, and rapid as entire process of screening requires only five days. With these features, it could be useful for advancing breeding, generation and reducing the number of breeding

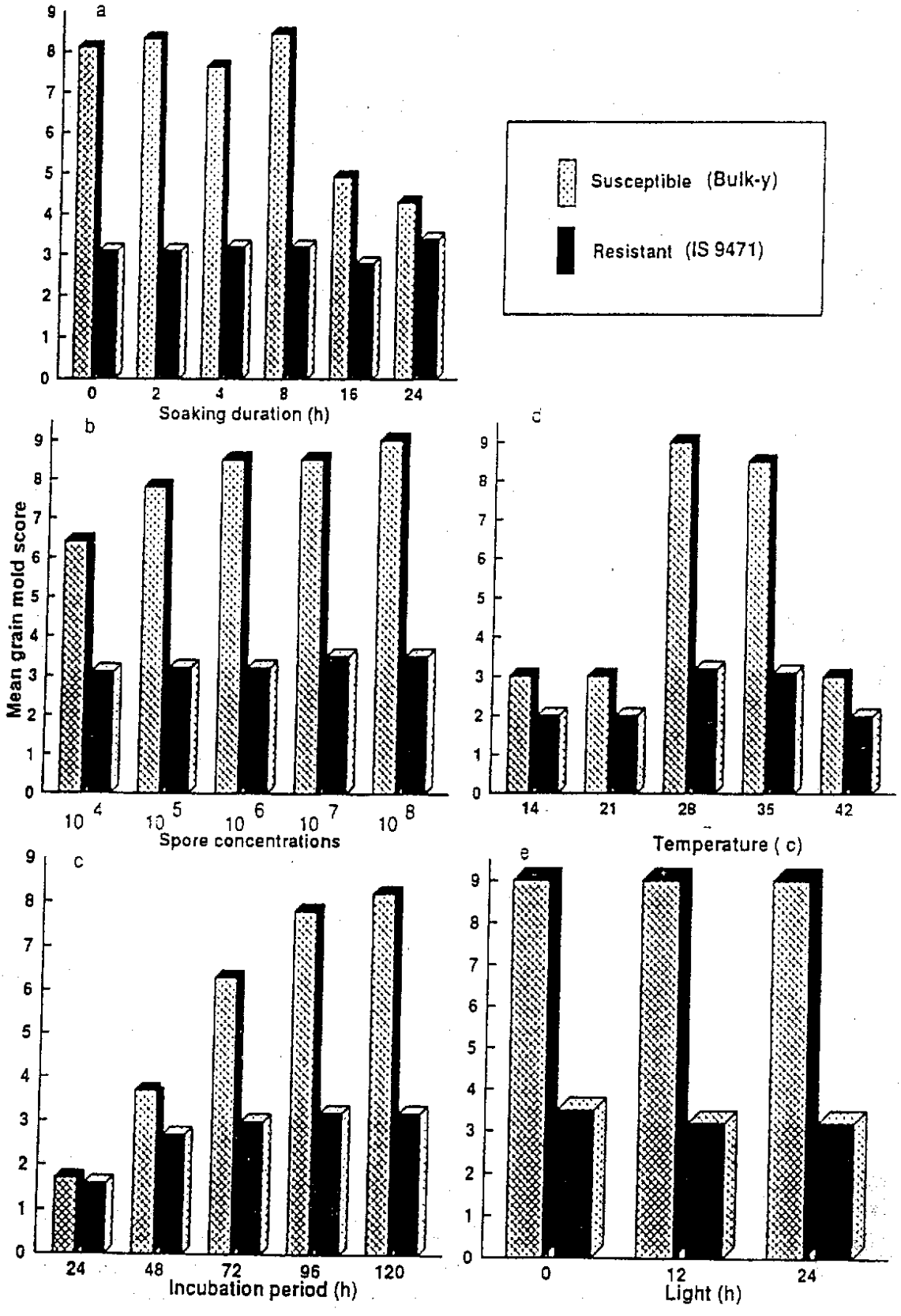

Fig. 1. Effect of (a) soaking duration (h), (b) spore concentration, (c) Incubation period (h), (d) Temperature (c) and (e) light on grain mold colonization

material for field screening. Similar, reports are available on pearl millet downy mildew caused by *Sclerospora graminicola* (Singh and Gopinath 1985; and Singh, *et al.*, 1997).

By using this technique, resistance to individual moulds could be identified, which is not possible under field conditions.

High level of resistance observed in few accessions indicated that selected photoperiod sensitive germplasms would likely to be an excellent sources of mould resistance. However, there is a need to further evaluate such genotypes extensively on multi-location basis.

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