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# Host plant resistance to grain mould in germplasm accessions of pearl millet (*Pennisetum glaucum* [L.] R. Br.)

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**Abstract**
The paucity of information on the moulds in Indian pearl millet (*Pennisetum glaucum*) led to the studies that were conducted at ICRISAT, India to evaluate (a) 447 germplasm accessions of 32 countries for mould reaction in rainy season, (b) threshed grain mould rating (TGMS) and mycoflora on grains of each accession, and (c) mould scores in field and *in vitro*. Post physiological maturity evaluation showed that 16% of the accessions secured a mould rating of 2. In TGMS, 18% were mould free and 57% secured a rating of 2 on a 1–9 scale. Assessment of twenty representative accessions *in vitro* against individual and mixed conidial suspensions ($1 \times 10^{(6)}$ conidia $ml^{(-1)}$) of *Fusarium moniliforme, F. pallidoroseum* and *Curvularia pennisetti* indicated significant correlation ($r = 0.97$) between the overall field and *in vitro* scores of mixed spores inoculations. The mycoflora for TGMS in blotter test revealed that *Fusarium moniliforme, F. pallidoroseum, Curvularia pennisetti, Helminthosporium* spp., *Alternaria* spp. and *Colletotrichum* spp. to be the major fungi affecting pearl millet grain. It is advisable to harvest panicles at the physiological maturity stage to obtain better quality grains. A strong negative correlation between TGMS and % GS ($r = 0.4601$) and positive correlation between TGMS and % UGS ($r = 0.4654$) indicated that, the lesser the threshed grain mould rating higher the % seed germination.

**Keywords:** *Pearl millet, germplam accession, host plant resistance, grain mould*

## Introduction

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is an important warm-season cereal grown primarily for grain production in some of the most marginal environments in the arid and semi-arid tropical (SAT) regions of Asia and Africa. India is the largest producer of pearl millet in the world, in terms of both the area and production. In India, about 87% of the area is in the states of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana with important production areas also in Karnataka, Andhra Pradesh and Tamil Nadu in the south. The pearl millet area in India varied from 9.03 m ha (2000–2004) to 10.71 m ha during 1990–1995 indicating a declining trend. But production

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increased from 6.49 m t in 1990–1995 to 6.67 m t during 2000–2004. Significant increment in productivity from 634 kg/ha in 1990–1995 to 730 kg/ha in 2000–2004 compensated for the decline.

Grain mould, or head mould of bajra, is also one of the leading problems during monsoon and most of the grain moulds, mainly biotrops in general and saprophytes in particular, gain entry into the mature seeds just after rains. Mechanical injuries such as cracks, breakages in the seeds and insect damage also help in the entry of grain mould (Manoharachary 1988). There are few reports of field grain moulds causing significant damage in pearl millet, although many have been isolated from pearl millet grain (Sharma & Basuchoudhary 1975, Shetty et al. 1982, Yadav 1978). The most frequently reported genera are *Helminthosporium rostrata* and *Curvularia lunata*. Ramakrishnan (1971) cites one report *Cladosporium herbarum* causing mouldiness and uneven grain development in pearl millet in southern Africa.

The result of grain mould infection is the loss in grain production similar to sorghum head mould (Williams Rao 1978). Wiehe (1953) noticed uneven grain production in bajra earheads infected by *C. herbarum*. Positive correlation was observed in bajra between carbohydrate and degree of deterioration due to fungi. Onesirosan (1975) reported *Fusarium* spp. as responsible for the head mould of bajra in southern Nigeria. Mathur et al. (1960) observed *Curvularia penniseti* to be associated with head mould of batra. Raveesha et al. (1985) found *Drechslera setariae, Cladosporium cladosporoides, Phoma exigua, Alternaria* spp. and *Fusarium* sp. are major fungal components of mouldy grains. They also isolated head mould pathogens from basal senescent leaves, plant debris and soil. From these sources the fungal propagules get air-borne and bring about earhead infection. An *in vitro* screening technique for screening mould fungi in sorghum has been developed by Singh and Navi (2001), which can be applied to pearl millet *in vitro* screening. *Fusarium moniliforme*, *F. pallidoroseum, C. pennisetti, Helminthosporium* spp., *Alternaria* spp. and *Colletotrichum* spp. are the major fungi affecting pearl millet grain in India (Navi & Tonapi 2004).

Manoharachary and Kunwar (1991) reported an association of fungi with bajra at pre-flowering and at harvest stages. Williams and McDonald (1983) have suggested identifying sources of resistance to grain mould in germplasm collections and new cultivars for the capacity to resist or avoid field fungi to resist factors such as insect pests that predispose the grain to mould development. In the present study, attempts were made to identify resistant sources, which are environment friendly and is one of the major components of integrated disease management (IDM) approach.

## Material and methods

### *Plant material*

A total of 525 pearl millet germplasm accessions were exclusively planted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Asia Center (IAC), Patancheru, Andhra Pradesh (AP) to identify resistance to downy mildew disease. Of which 447 accessions (including 1 hybrid) originating from Algeria (3), Benin (3), Botswana (4), Burkina Faso (31), Cameroon (18), Chad (16), United Kingdom (1), Ghana (51), ICRISAT (4), India (93), Kenya (4), Mali (48), Morocco (2), Malawi (4), Mozambique (3), Namibia (2), Niger (66), Nigeria (15), Pakistan (4), Russia (1) Senegal (11), Somalia (3), South Africa (9) Sudan (11), Tanzania (2), Togo (6), Tunisia (2), Uganda (7) USA (7), Yemen (1), Zambia (7), and Zimbabwe (8) were made use of in the present study.

*Isolation and identification of grain mould fungi*

Mature grains of a panicle showing infection by various grain mould fungi, were selected (based on colonization color by the fungi) and surface-sterilized in a 0.1% mercuric chloride ($HgCl_2$) for 2 min, rinsed thoroughly in sterile distilled water (SDW) and transferred aseptically on to potato dextrose agar medium (Himedia Laboratories Pvt. Ltd., Bombay) in Petri dishes at 3 seed per dish. The plates were incubated at $25 \pm 2^{\circ}C$ for 8–10 days. The fungi growing from the grains were sub-cultured on PDA. Based on colony characters, shape and size of the fungal spores fungi were identified (Booth 1971).

In vitro *screening*

*Multiplication of inocula.* About 20 g grain was taken separately for each fungus in a 100 ml conical flask and soaked in SDW for 3 h. The excess water was drained out. Soaked grain were sterilized at 121.6°C, 15 lbs pressure PSI, for 15 min. The flasks were allowed to cool in the laboratory at $25 \pm 2^{\circ}C$ before inoculating separately with individual fungus (*F. moniliforme, F. pallidoroseum,* and *C. penniseti*) under aseptic conditions. The flasks were incubated at 25–30°C for 8–10 days. The flasks were then stored at $4 \pm 1^{\circ}C$ until the infected grains were used for inoculum.

*Preparation of inocula for* in vitro *screening.* A few grains colonized by different fungi were transferred separately to a 25-ml beaker containing about 10 ml SDW. The beaker was shaken thoroughly on a Vortex mixer and the spore suspension was filtered through a stainer. Concentration of the suspension was adjusted to $1 \times 10^5$ $ml^{-1}$ SDW.

In vitro *screening.* This technique is to identify resistance sources to pearl millet grain mould under laboratory conditions. The technique was followed as mentioned here. Surface sterilized 25–30 seeds of a test entry dipped separately in a spore suspension of *F. moniliforme, F. pallidoroseum,* and *C. penniseti* and their mixture. The grains were transferred into a sterilized moist chamber (lower lid of Petri plate lined with a thin layer of cotton, followed by a blotting paper, and 10–15 ml water and autoclaved at 121.6°C temperature, 15 lbs pressure per square inch for 15 min under aseptic conditions). The chambers were incubated at $25 \pm 2^{\circ}C$ for 4–5 days, and were scored for grain mould resistance using hanging armed magnifying lens with fluorescent light on a 1–9 scale where, 1 = No mould, 2 = Up to 5% surface area of grains moulded, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75%, and 9 = >75% surface area of grains moulded.

*Field screening*

For the present study accessions were not exclusively planted for grain mould evaluation. However, these were planted to identify resistance to downy mildew disease (*Sclerospora graminicola*) at IAC, Patancheru (AP). The accessions were planted in a single replication each with 4 m length plot. In the agronomy of the crop, fertilizer di-ammonium phosphate (DAP) 100 kg/ha was the basal dose and urea 100 kg/ha for top dressing. The crop was given furrow-irrigation once in a week.

*Provision of inoculum and favorable environment.* Natural inoculum was relied upon for the mould development during the rainy season. Weather data (Figure 1) was recorded from 50% flowering to 2 weeks after physiological maturity, as rainfall, humidity and temperature are

important in the grain mould development because the entries in the nursery have different flowering dates. However, high moisture levels were assumed by rains that lead to elevated humidity regimes (a total of 22 rainy days during the period from 50% flowering to 2 weeks after physiological maturity during 1994) and this information (Figure 1) was used to interpret differences in the reaction among the test entries.

*Observations.* Days to 50% flowering was recorded when approximately 50% plants in a plot had flowered and five plants at random in each plot were tagged for further observations. Grain mould scoring tagged plants (during 50% flowering record) were selected and were rated for mould development once, at grains reach physiological maturity (black layer formation) and then 14 days after physiological maturity. During the second record, five panicles from each of the accessions were harvested and assessed for grain mould colonization using magnifying lens with fluorescent light. Five panicles of each accession were scored for colonization by an individual fungus and also by a combination of several fungi, following the 1 – 9 rating scale. For threshed grain mould score, after the second observation of the 5 panicles of each accession the same were threshed and evaluated on the 1 – 9 scale under the lens.

*Assessment of seed mycoflora.* To identify and assess different fungi on field harvested grains of pearl millet, about 200 grains from each accession were plated separately in plastic dishes containing moistened blotting paper and allowed for germination in an incubator at 25 $\pm$ 1°C with 12 h cycle light. Three days after plating grains were observed for fungal colonization both on germinated and ungerminated grains under WILD M8 Discussion Steromicroscope and were evaluated on the 1 – 9 scale using magnifier lens. A Photomicrograph of different fungi observed during the study was taken on a Fluorescence microscope (Olympus Vanox). Percent seed germination and percent germinated seeds colonized by individual fungus were calculated. Similarly for the percentage of seed ungermination and their colonization by different fungi was assessed and analysed.

*Statistical analysis*

Mean grain mould scores of *in vitro* screening technique and field score were calculated and correlated with one another. Mean grain mould scores of the panicles and mean

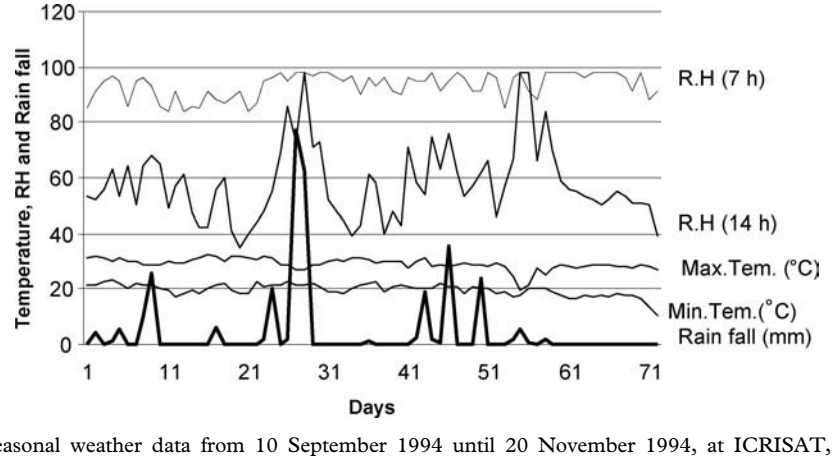


Figure 1. Seasonal weather data from 10 September 1994 until 20 November 1994, at ICRISAT, Patancheru, Andhra Pradesh, India, from 50% flowering to two weeks after physiological maturity.

threshed grain mould score of each accession were calculated and correlated with days to flowering. Percent seed germination and the percentage of germinated and ungerminated seeds colonized by different fungi was calculated and correlated with threshed grain mould score.

## Results and discussion

### *Isolation and identification of fungi*

Major grain mould fungi appeared in field were isolated on potato dextrose agar medium (based on symptom type) were *Fusarium moniliforme, F. pallidoroseum, C. penniseti*. Colony characters *F. moniliforme* was white, pluffy growth, *F. pallidoroseum* was pink and that of *C. penniseti* was black in color. Identification of other fungi during assessment for seed mycoflora was based on fungal growth on the seed surface (Figure 2). During the test, seeds showing different colors on their surfaces were examined under the steromicroscope. Seeds infected by *Helminthosporium* sp. produced a hairy or velvety olive brown to black growth. Conidia were pale brown cylindrical slightly curved or rarely straight.

The *Alternaria*-infected seeds produced wooly or powdery chains of dark brown conidia of variable lengths and shapes. Hyphae were dark brown, thick septate and branched. Conidiophores were simple erect and often clustered. They produced darkly pigmented conidia in an acropetal succession of simple or brached chains. The chains normally branch at the beak of the spore of some times from the short lateral projections of the beak. Conidia had transverse and oblique septa and were ovoid to obovoid, obclavate, pyriform, ellipsoidal, muriform with an elongated terminal cell. Conidia often showed a short conical or cylindrical beak which may be up to one third the length of the conidium.

Seeds colonized by *Colletotrichum* sp. had dark brown to black acervuli scattered on their surface. These acervuli were irregular in shape and consisted of dark setae. Sometimes acervuli were also formed on the glumes. On incubated seeds the fungus produced numerous acervuli. The acervuli consisted of a gelatinous or mucoid salmon orange colored conidial mass. Conidiophores were hyaline. Conidia were hyaline. Setae were brown with dark swollen tip. In some cases macroconidia of *Fusarium* sp. were observed.

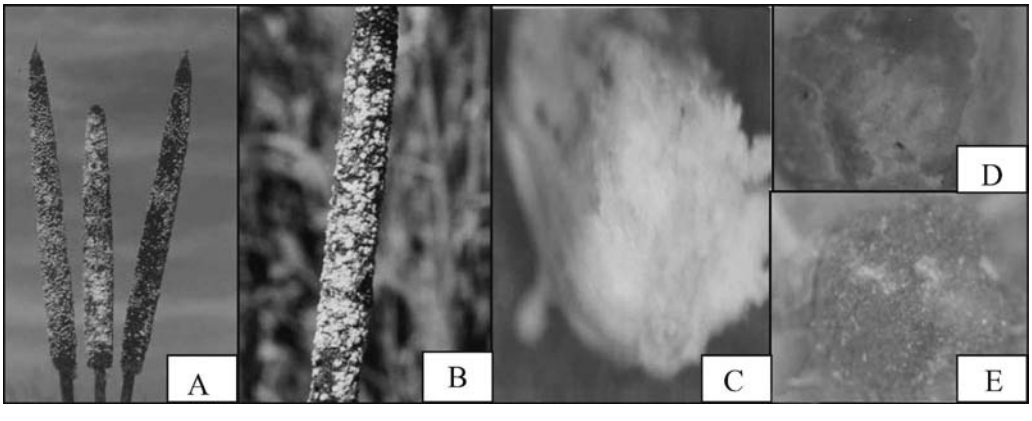


Figure 2. (A and B). Mould growth on pearlmillet panicles. (C) Fungal colonization by *Fusarium Moniliforme*. (D) Fungal colonization by *F. pallidoroseum*. (E) Fungal colonization by *Curvularia pennisetti.*

### In vitro *and field screening*

Although no screening techniques were accessible to identify sources of resistance to pearl millet grain mould, only 20 accessions were evaluated for grain mould resistance, of which 2 were in score 2, 3 in score 4, and 2 in score 4 against mixed spore suspension of *F. moniliforme*, *F. pallidoroseum* and *C. penniseti* (Table I).

However, grain mould score of each of the 20 accessions against individual fungus both in the *in vitro* technique and in the field were correlated (Table II). Field scores of *F. pallidoroseum*, *C. Penniseti* and overall score of panicles were significantly correlating with *in vitro* scores at 1% level of significance. General score in the field also correlated (at 1% level of significance) with the *in vitro* score of mixed spore suspension of the accessions ($r = 0.9759$).

In nature, different fungi causing grain mould occurs. Sincere efforts were made to match colonization of grains in the field to that of *in vitro* screening by using mixed spore suspension. Using the suspension accessions under the test were evaluated following dip inoculation technique as stated in material and methods. In general, significant correlation was observed between grain mould scores of lab and field at 1% level of significance. Therefore, it would be an appropriate technique to identify sources of resistances to pearl millet grain mould. This technique would also help save: (i) resources like land, labor and money, and (ii) time and

Table I. Mean grain mould score of 20 pearl millet germplasm accessions (IP Nos.) evaluated for resistance for both under *in vitro* and field conditions during the rainy season 1994*.

| | | Lab score | | | | Field score | | | |
|---|---|---|---|---|---|---|---|---|---|
| IP Nos. | Country | FM | FP | CP | MIX | FM | FP | CP | MIX |
| 3220 | India | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 2 |
| 3327 | India | 2 | 2 | 3 | 4 | 1 | 1 | 3 | 3 |
| 3543 | India | 2 | 2 | 3 | 3 | 1 | 1 | 2 | 2 |
| 3990 | India | 2 | 2 | 3 | 3 | 1 | 1 | 2 | 2 |
| 4125 | India | 2 | 2 | 5 | 6 | 1 | 2 | 5 | 6 |
| 4179 | India | 2 | 2 | 2 | 3 | 1 | 1 | 2 | 3 |
| 4185 | India | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 |
| 4417 | India | 2 | 2 | 3 | 4 | 1 | 1 | 2 | 3 |
| 4830 | India | 2 | 2 | 4 | 6 | 2 | 2 | 4 | 6 |
| 6344 | Mali | 1 | 3 | 4 | 7 | 1 | 3 | 4 | 7 |
| 6377 | Mali | 2 | 2 | 5 | 9 | 1 | 2 | 6 | 8 |
| 9366 | Ghana | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 2 |
| 11650 | UK | 2 | 5 | 6 | 9 | 1 | 4 | 6 | 9 |
| 13520 | India | 2 | 3 | 5 | 8 | 1 | 3 | 6 | 8 |
| 13873 | Burkina Faso | 2 | 3 | 6 | 9 | 1 | 3 | 6 | 8 |
| 13904 | Burkina Faso | 2 | 3 | 7 | 9 | 1 | 3 | 7 | 9 |
| 17484 | Chad | 2 | 3 | 4 | 6 | 1 | 3 | 3 | 4 |
| 18193 | Mali | 1 | 4 | 5 | 9 | 1 | 4 | 4 | 8 |
| 19615 | Niger | 2 | 3 | 4 | 8 | 1 | 3 | 4 | 7 |
| Hb 3 | India | 3 | 2 | 7 | 9 | 1 | 2 | 7 | 8 |
| | Mean | 1.85 | 2.50 | 4.10 | 5.95 | 1.05 | 2.10 | 3.95 | 5.35 |
| | SD | 0.48 | 0.87 | 1.58 | 2.64 | 0.22 | 1.04 | 1.80 | 2.67 |
| | SE± | 0.11 | 0.19 | 0.35 | 0.59 | 0.05 | 0.23 | 0.40 | 0.60 |

*on 1–9 scale where: 1 = no mould; 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75% and 9 = >75% mould; FM, *Fusarium moniliforme*; FP, *F. pallidoroseum*; CP, *Curvularia penniseti*; MIX, mixture of FM, FP and CP; GSC, General mould score in the field.

energy. The technique would also help screen test material under controlled conditions during any season of the year colonization by individual fungus.

The relationship between mean grain mould scores of physiological maturity (PM) and 14 days after PM (PM1), was highly correlating at 1% level of significance ($r = 0.6751$). Similarly PM1-score was significantly correlating with scores of *F. pallidoroseum* and *C. penniseti* with the '*r*' values; 0.6277 and 0.8742, respectively (Table III).

During the study it was observed that, at PM-stage record 50 accessions were completely free from colonization, 353 in score 2 (1–5% mould) and 44 in score 3 (6–10% mould). Whereas in the PM1-stage record (overall score of different fungi involved in colonization) the values were; 0.71, and 151 respectively. However, none of the accessions were in score 4 and none in score 1, and about 10% each were in score 5 (38) and score 6 (44) and only 5% (24 accessions) were between score 6 and 9 (Table IV).

When each of the accessions were examined closely for colonization by individual fungus (Figure 2) under the magnifying lens, 4445 accessions were found to be free from colonization by *F. moniliforme*, 193 with *F. pallidoroseum*, and none with *C. penniseti*. This would indicate that, at least in the visual score, the predominant fungus observed on grains was *C. penniseti* followed by *F. pallidoroseum*. However, 240 accessions were

Table II. Correlation matrix for mean grain mould score of 20 accessions screened both under *in vitro* technique and field screening during the rainy season 1994.

| | | | | | | | | | Df = 18 |
|---|---|---|---|---|---|---|---|---|---|
| FFM | 1 | 1.0000 | | | | | | | |
| FFP | 2 | −0.0220 | 1.0000 | | | | | | |
| FCP | 3 | 0.0064 | 0.6670 | 1.0000 | | | | | |
| FGSC | 4 | 0.0559 | 0.8485 | 0.9286 | 1.0000 | | | | |
| LFM | 5 | 0.0721 | −0.0703 | 0.3985 | 0.1983 | 1.0000 | | | |
| LFP | 6 | −0.1325 | 0.8848** | 0.4646* | 0.6596** | −0.0605 | 1.0000 | | |
| LCP | 7 | −0.0145 | 0.7223** | 0.9512** | 0.9056** | 0.4185 | 0.5488 | 1.0000 | |
| MIX | 8 | 0.0044 | 0.8558** | 0.9047** | 0.9759** | 0.2327 | 0.6681 | 0.9148 | 1.0000 |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

*Correlation at 5% level of significance; **correlation at 1% level of significance; FFM, FFP and FCP are grain mould score in the field against *F. moniliforme*, *F. pallidoroseum* and *C. penniseti*, respectively; FGSC is overall grain mould score in the field, LFM, LFP, LCP; MIX are grain mould scores in the *in vitro* technique against the above fungi and their mixture.

Table III. Correlation matrix for grain mould score in the field and laboratory.

| | | | | | | | | Df = 445 |
|---|---|---|---|---|---|---|---|---|
| DTFP | 1 | 1.0000 | | | | | | |
| PM | 2 | 0.0484 | 1.0000 | | | | | |
| PM1C | 3 | 0.0431 | 0.6751** | 1.0000 | | | | |
| Fm | 4 | −0.0333 | 0.0865* | 0.0137 | 1.0000 | | | |
| Fp | 5 | 0.0017 | 0.4280** | 0.6277** | 0.0523 | 1.0000 | | |
| Cp | 6 | 0.0439 | 0.6332** | 0.8742** | 0.0132 | 0.3687 | 1.0000 | |
| TGMS | 7 | −0.0773 | 0.2848** | 0.3665** | 0.0387 | 0.2705** | 0.3249** | 1.0000 |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

*Correlation at 5% level of significance; **correlation at 1% level of significance; DTF, Days to 50% flowering; PM, Grain mould score at physiological maturity; PM1, Grain mould score 14 days after PM with general score and for individual fungus like Fm, *F. moniliforme*; Fp, *F. pallidoroseum*; Cp, *C. penniseti*; TGMS, Threshed grain mould score.

Table IV. Summary of 447 pearl millet germplasm accessions evaluated for grain mould resistance on 1–9 scale during the rainy season 1994.

| Scale | Colonization (%) | PM | PM1 | PM1* | | | TGMS |
|---|---|---|---|---|---|---|---|
| | | | | FM | FP | CP | |
| 1 | 0 | 50 | 0 | 445 | 193 | 0 | 81 |
| 2 | 1–5 | 353 | 71 | 2 | 240 | 115 | 252 |
| 3 | 6–10 | 44 | 151 | 0 | 12 | 207 | 74 |
| 4 | 11–20 | 0 | 119 | 0 | 2 | 75 | 29 |
| 5 | 21–30 | 0 | 38 | 00 | 0 | 29 | 6 |
| 6 | 31–40 | 0 | 44 | 0 | 0 | 16 | 5 |
| 7 | 41–50 | 0 | 9 | 0 | 0 | 5 | 0 |
| 8 | 51–75 | 0 | 10 | 0 | 0 | 0 | 0 |
| 9 | >75 | 0 | 5 | 0 | 0 | 0 | 0 |

PM, mould score at Physiological maturity; PM1, mould score 14 days after PM; *mould score against individual fungus; FM, *F. moniliforme*; FP, *F. pallidoroseum*; CP, *C. penniseti*; TGMS, Threshed grain mould score.

colonized by *F. pallidoroseum* in score 2, 12 in score 3, and 2 in score 4. The values of *C. penniseti* were 115, 207, and 75 respectively. In the field screening none of the accessions colonized by *F. pallidoroseum* were in score 5 and above whereas, in case of *C. penniseti* 50 accessions were in colonization score between 5 and 7. No accessions were colonized by *C. penniseti* in score 8 and 9. In this context it is important to note that, not only are the numbers of fungi involved important but also their severity on the entry under the test is important. Similarly the stage at which colonization appears is important. From the study it is evident that the maximum number of accessions was highly resistant (between score 1 and 3) at PM-stage record compare to PM1-stage record. Therefore from the evidence available from this experiment it is advisable to harvest panicles at the physiological maturity stage. Manoharachary and Kunwar (1991) reported presence of fungi right from the pre-flowering stage to harvest. However, the severity of the mould was not reported.

*Threshed grain mould score*

Of the 447 accessions evaluated for threshed grain mould score, 81 were completely free from mould (0% colonization), 252 were in score 2, 74 in 3, 29 in 4, 6 in score 5 and 5 in score 6. However, none of the accessions were between score 7 and 9 (Table IV). Nearly 57% of the accessions were in score 2, 17% in score 3, 6% in score 4 and <2% in score 5 and 6. There is lot of difference in the mould score of PM1-stage and threshed grains. In the former none of the accessions were found free from colonization and only 71 were in score 2 and 161 in score 3, whereas, the number of accessions in the scores after threshing were 81, 252 and 74, respectively. Possible reasons could be washing away of fungal growth during threshing. This would, however, give a wrong impression to the grower that the seed material is good. The source of inoculum in the following season could be the presence of fungal bodies inside or outside the seed coat. Upon threshing, moulded and healthy seeds could be easily identified. Therefore, an appropriate method to test the presence of fungi inside or outside the seed-coat is the moist blotter test. With the test, one could identify the presence of fungal bodies (spores/mycelium) possibly through the stimulation generated by high relative humidity in the moist

blotting plate. The relationship among scores of PM, PM1, individual fungus (except *F. moniliforme*) and threshed grains was significantly correlating at 1% level of significance (Table III).

*Assessment of seed mycoflora*

All the 447 accessions evaluated for percentage of seed germination. The percentage of germinated and ungerminated seeds colonized by *F. moniliforme*, *F. pallidoroseum, C. penniseti, Helminthosporium* sp. *Alternaria* sp., and *Colletotrichum* sp. were assessed for percentage of colonization on the 1–9 scale. Of the 447 accessions originating from 32 countries, 294 accessions had percentage of germination between 76 and 100, 67 in 51–75%, 14 in 41–50%, 35 in 31–40%, 11 in 21–30%, 13 in 11–20%, 3 in 6–10% and only one in 1–5% germination stage. However, nine accessions (IP Nos. 6217 of Cameroon, 6497 of Mali, 7497 of Somalia, 7953 of India, 8870 and 9300 of South Africa, 9427 of Ghana, 12183 of Nigeria and 17997 of USA) had no germination. The percentage of ungermination of 447 accessions assessed, 32 were between 76 and 100%, 53 in 51–75%, 11 in 41–50%, 42 in 31–40%, 43 in 21–30%, 114 in 11–20%, 53 in 6–10%, 38 in 1–55 and 61 in 0% germination.

From the study it was observed that only 7 accessions (IP Nos. 6475 of Mali, 6603 of Malawi, 9948 of Zambia, 10463 of Zimbabwe, and 19634, 19635 and 19637 of Niger) were completely free from mould in the moist blotter test and in the threshed grain mould score. The germination of these accessions was between 80% and 100%.

The accessions assessed for seed mycoflora were correlated with threshed grain mould score (TGMS), percentage of seed germination (% GS), and percentage of ungermination (% UGS). There was a strong negative correlation between TGMS and % GS ($r = 0.4601$) and strong positive correlation between TGMS and % UGS ($r = 0.4654$) at 1% level of significance. This would clearly indicate that, the lesser the threshed grain mould score the higher the percentage of seed germination. It is also true that higher the threshed grain mould score the lower percentage of seed germination. This type of relationship should be acceptable because when there is a higher TGMS the % GS would be inhibited, possibly due to the toxic metabolites produced by fungi. This information is in agreement with Jain and Jain (1991) since the toxic metabolite of fungi causes a reduction in % seed germination. Positive correlation was observed between TGMs, where % germinated seeds colonization score (GSC) and % ungerminated seeds colonization (UGSC) were $r = 0.3777$ and 0.2581, respectively. However, correlation among other parameters is given in Table V.

For the percentage of germinated seed colonization there were about 200 accessions (45%) in score 2, 121 in score 3, and 41 in score 4 (Table VI). It is interesting to note that maximum number of accessions originating from India were highly resistant followed by Niger and Mali. Of the 45% accessions listed in score 2, 22% contribution alone is from India. This would mean India has got enough sources of resistances to pearl millet grain mould. In the % ungerminated seed colonization there were 320 accessions in score 9 and only 64 in score 1 and 15 in score 2 (Table VII).

During the survey conducted by Batsa and Tamang (1983) in Nepal, none of the bajra genotypes were found resistant to grain moulds. From this preliminary study it is advisable to make use of the resistant sources listed in this report in areas where sources of resistance are a problem, and where farmers cannot afford to use chemicals to control this disease. It is also advised to use these sources of resistance in breeding program. If we compare this information with TGMS, there is 12% reduction in number of accessions in

Table V. Correlation matrix for assessment of seed mycoflora in the blotter test.

Df = 445

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| TGMS | 1 | 1.0000 | | | | | | | | |
| %GS | 2 | −0.4601** | 1.0000 | | | | | | | |
| GFM | 3 | 0.0420 | −0.1094* | 1.0000 | | | | | | |
| GFP | 4 | 0.2230** | −0.1169** | 0.0477 | 1.0000 | | | | | |
| GCP | 5 | 0.2374** | −0.2176** | −0.0966 | −0.0011 | 1.0000 | | | | |
| GHL | 6 | 0.0013 | 0.0735* | −0.1323 | −0.0086 | 0.0824 | 1.0000 | | | |
| GAL | 7 | 0.1530** | −0.2299** | 0.0725 | 0.0729 | 0.1254 | −0.2336 | 1.0000 | | |
| GCO | 8 | 0.1710** | −0.1768** | −0.0559 | 0.9489 | 0.0352 | −0.0222 | 0.0397 | 1.0000 | |
| GSSC | 9 | 0.3777** | −0.2587** | 0.0515 | 0.3501 | 0.3048 | −0.0296 | 0.3595 | 0.3364 | 1.0000 |
| %UGS | 10 | 0.4654** | −0.9974 | 0.1093 | 0.1165 | 0.2164 | −0.0714 | 0.2242 | 0.1764 | 0.2607 |
| UGFM | 11 | −0.0041 | 0.0444 | 0.1655 | −0.0587 | −0.0871 | 0.0242 | −0.1016 | −0.0524 | −0.0679 |
| UGFP | 12 | 0.0407 | −0.0149 | 0.0063 | 0.0610 | −0.0316 | −0.0599 | −0.0553 | 0.0419 | 0.0372 |
| UGCP | 13 | −0.1088* | 0.1082 | −0.0197 | −0.0666 | −0.0043 | −0.0842 | −0.1107 | −0.1222 | −0.1230 |
| UGHL | 14 | 0.1038* | −0.2400 | −0.0013 | −0.0938 | 0.0702 | 0.0348 | −0.0979 | 0.0139 | −0.0057 |
| UGAL | 15 | 0.0688 | −0.2688 | 0.0556 | 0.0534 | −0.0438 | −0.1209 | 0.2929 | 0.0305 | 0.0955 |
| UGCO | 16 | 0.1486** | −0.1147 | 0.0163 | 0.0683 | 0.0937 | −0.0538 | 0.1596 | 0.1414 | 0.2467 |
| UGSC | 17 | 0.2581** | −0.3821 | 0.0826 | 0.0207 | 0.0384 | −0.1684 | 0.2055 | 0.1005 | 0.2100 |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| %UGS | 10 | 1.0000 | | | | | | | | |
| UGFM | 11 | −0.0490 | 1.0000 | | | | | | | |
| UGFP | 12 | 0.0141 | −0.0069 | 1.0000 | | | | | | |
| UGCP | 13 | −0.1078* | −0.0796 | −0.1894 | 1.0000 | | | | | |
| UGHL | 14 | 0.2346** | −0.0036 | −0.1146 | −0.0477 | 1.0000 | | | | |
| UGAL | 15 | 0.2745** | −0.1445 | −0.0512 | −0.0858 | −0.2587 | 1.0000 | | | |
| UGCO | 16 | 0.1164* | −0.0526 | −0.0517 | −0.0895 | −0.1311 | −0.1204 | 1.0000 | | |
| UGSC | 17 | 0.3857** | 0.0478 | 0.2116** | 0.1825 | 0.2079** | 0.4457** | 0.2106 | 1.0000 | |
| | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | |

*Correlation at 5% level of significance; **Correlation at 1% level of significance; TGMS, Threshed grain mould score; % GS, % seed germination; GFM, GFP, GCP, GHL, GAL, and GCO are % germinated seeds colonized by grain mould fungi *F. moniliforme*, *F. pallidoroseum*, *C. penniseti*, *Helminthosporium* sp. *Alternaria* sp. and Colletotrichum sp. respectively; Similar data for ungerminated seeds (UG).

Table VI. Summary of mean grain mould score of 447 pearl millet germplasm accessions (originating from 32 countries) evaluated for ''seed mycoflora of germinated seeds'' in the moist blotter test.

| | No. of accessions in grain mould score 1–9 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Country | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Algeria | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Benin | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| Botswana | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Burkina Faso | 0 | 10 | 5 | 5 | 5 | 3 | 0 | 1 | 2 |
| Cameroon | 1 | 11 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chad | 0 | 6 | 6 | 2 | 2 | 0 | 0 | 0 | 0 |
| Ghana | 1 | 11 | 14 | 11 | 1 | 4 | 1 | 0 | 10 |
| ICRISAT | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| India | 1 | 45 | 29 | 6 | 5 | 3 | 0 | 1 | 3 |
| Kenya | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Malawi | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mali | 2 | 24 | 13 | 5 | 3 | 0 | 0 | 0 | 1 |
| Morocco | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mozambique | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Namibia | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Niger | 4 | 32 | 21 | 2 | 4 | 1 | 1 | 0 | 1 |
| Nigeria | 1 | 6 | 6 | 0 | 2 | 0 | 0 | 0 | 0 |
| Pakistan | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Russia | 0 | 01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Senegal | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somalia | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| South Africa | 2 | 3 | 1 | 3 | 1 | 0 | 0 | 0 | 0 |
| Sudan | 0 | 3 | 3 | 2 | 3 | 0 | 0 | 0 | 0 |
| Tanzania | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Togo | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| Tunisia | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| USA | 1 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 |
| Uganda | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| United Kingdom | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Yemen | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zambia | 1 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zimbabwe | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 17 | 203 | 121 | 41 | 30 | 12 | 2 | 2 | 19 |

score 2. That means there must be at lease trace amount of fungal bodies on the seed surface or inside the seed coat which we may not detect in the visual scoring. The growth of the same fungi could be exposed in the blotter test, whereas numbers of accessions colonized by different fungi in the blotter test were 12 in score 3, compared to 74 of threshed grains, which indicates increased colonization of the accessions. This is possible because the growth of fungi or appearance of fungal bodies on seed surface was missed or not observed in visual score, or the growth might have been expressed when congenial condition for the fungi prevailed in the moist blotter test. In some accessions like IP 6217 there was absolutely no germination possibly due to colonization by several fungi. Indirect effect could also be due to toxic metabolites produced by the fungi. In contrast, some of the accessions like IP 6603 had 100% germination with no colonization by any of the fungi stated above. The predominant fungi observed during the study were species of *Helminthosporium*, *Alternaria*, *Curvularia*, *Colletotrichum* and *Fusarium*.

Table VII. Summary of mean grain mould score of 447 pearl millet germplasm accessions (originating from 32 countries) evaluated for ''seed mycoflora of ungerminated seeds'' in the moist blotter test.

| | No. of accessions in grain mould score 1–9 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Country | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Algeria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Benin | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Botswana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| Burkina Faso | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 25 |
| Cameroon | 3 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 11 |
| Chad | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 14 |
| Ghana | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 43 |
| ICRISAT | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| India | 14 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 76 |
| Kenya | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Malawi | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Mali | 6 | 2 | 1 | 1 | 2 | 1 | 0 | 1 | 34 |
| Morocco | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Mozambique | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Namibia | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Niger | 9 | 6 | 2 | 1 | 4 | 2 | 0 | 0 | 42 |
| Nigeria | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 12 |
| Pakistan | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Russia | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Senegal | 4 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 1 |
| Somalia | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| South Africa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 |
| Sudan | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| Tanzania | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Togo | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 |
| Tunisia | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| USA | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Uganda | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| United Kingdom | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Yemen | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zambia | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 |
| Zimbabwe | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 4 |
| Total | 64 | 15 | 11 | 6 | 12 | 10 | 3 | 6 | 320 |

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