# Genetic Resistance to Pearl Millet Downy Mildew II. Resistance in Wild Relatives

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**Abstract**

Genetic resistance is the most economic and feasible method for control for downy mildew (DM) (*Sclerospora graminicola*) of pearl millet (*Pennisetum glaucum*). To identify genes for DM resistance with diverse origin, we tested 539 accessions of 12 wild *Pennisetum* species from 17 countries, in the greenhouse and field–disease nurseries. A total of 223 accessions were found DM free in all the tests. *P. violaceum* was the most susceptible of all the species, both for the frequency of susceptible accessions (93% accessions with >10% DM) and for the level of susceptibility (accessions showing up to 94% DM). Freedom of most of the *P. schweinfurthii* accessions from DM, coupled with resistance to rust (*Puccinia penniseti*), is encouraging because of its cross–compatibility with pearl millet. DM resistance genes from these wild species will be useful in the control of this disease, if found different from those of pearl millet. Successful cross–inoculation of pearl millet with sporangia produced on five wild species (*P. violaceum*, *P. mollissimum*, *P. purpureum*, *P. padicellatum*, and *P. polystachyon*) shows that these species may be serving as collateral/alternative hosts and also helping the pathogen in creating pathogenic variability. As these species are present in all pearl millet growing areas, a precise information on their role under farmers' field conditions is necessary for the development of cultivars with durable DM resistance.

**Key words:** *Pennisetum* spp, downy mildew, genetic resistance

बाजरे *(पेनिसिटम ग्लुकम)* के मृदु रोमिल (DM) *(स्क्लेरोस्पोरा ग्रेमीनीकोला)* के नियन्त्रण के लिए आनुवंशिकी प्रतिरोधकता आर्थिक और उपयुक्त रूप से उत्तम विधि है। DM प्रतिरोधकता की पहचान करने के लिए 17 देशों से प्राप्त 12 वन्य *पेनिसिटम* स्पीसीज की 539 एक्सीसियन्स की ग्रीन हाऊस और रोग ग्रस्त खेत नर्सरीयों में परख की गई। कुल 223 एक्सीसियन्स सभी परीक्षणों में DM से मुक्त पाई गई। सभी स्पीसीज में से वायोलेसियम दोनों आवृत्ति एक्सीसियन ग्राह्यता (93% एक्सेसियन्स >10% DM के साथ) और ग्राह्यता स्तर (एक्सेसियन्स 94% तक DM प्रदर्शन करने वाली) के लिए सर्वाधिक ग्राह्य स्पीसीज थी। *पी.स्वेनफुर्थि* एक्सीसियन की DM मुक्ति से तथा रोली रोधकता गुण के साथ युग्मित होना उत्साहवर्धक है क्योंकि इससे इसकी बाजरा के साथ पर सुसंगतता है। इस वन्य स्पीसीज की DM प्रतिरोधकता के जीन यदि बाजरा से भिन्न पाए गए तो इस रोग को नियन्त्रण करने में उपयोगी होंगे। पाँच वन्य स्पीसीज *(पी. वायोलेसियम, पी. मोलीसीमम, पी. परपुरियम, पी. पेडीसिलेटम* और *पी. पोलीस्टेक्योन)* पर उत्पादित स्पोरेन्जिया का बाजरा में सफल पर संरोपण यह दर्शाता है कि ये स्पीसीज बहि विकल्पत: परपोषी का काम करने के साथ साथ रोगाणु मुें रोग जनक परिवर्तिता निर्माण करने मे भी सहायक है। चूंकि ये स्पीसीज सभी बाजरा के क्षेत्रों में पूर्णतया पाई जाती है, इनके बारे में कृषक के खेत की परिस्थितियों में सशक्त ब्यौरा DM से दार्घविधि के लिए रोग प्रतिरोधक कृषिजोपजाति विकसित करने के लिए आवश्यकीय है।

Downy mildew (DM) [*Sclerospora graminicola* (Sacc.) Schröt.] is the most widespread and destructive disease of pearl millet [*Pennisetum glaucum* (R.) Br.] in India and western Africa (Singh, 1994; Singh *et al.*, 1993). In western Africa, where improved pearl millet cultivars are not yet widely grown, the disease causes substantial yield losses even in landrace cultivars (unpublished).

The disease has been successfully controlled in India by the use of resistant cultivars which is the most economic and environmentally–appropriate method. However, due to highly adaptive nature of the pathogen (Singh and Singh 1987), genetically uniform cultivars succumb to the disease within 3–5 years of their large scale cultivation (Safeeulla, 1977; Singh, 1994, and Singh *et al.*, 1987). In order that these cultivars hold their resistance to DM for a longer period of time, they must possess a broad genetic base. To achieve this, many different resistance genes are needed. Wild

relatives of pearl millet which have been exposed to the pathogen for centuries and so far remained unexploited, are likely to be a good sources of diverse genetic resistance. In this paper, we have discussed results of testing wild species to pearl millet DM and their implications for the long term control of this disease.

## Materials and Methods

A total of 539 accessions of 12 wild *Pennisetum* species from 17 countries (Table 2), were tested for their reaction to DM in the greenhouse and field–disease nurseries from 1992 to 1995. Seeds were obtained from the Genetic Resources Division (GRD) of the ICRISAT.

**Preliminary greenhouse screening.** Each accession was sown in two, 15 cm diameter plastic pots (about 50 seeds per pot), filled with a potting mixture consisting of alfisol, sand, and farmyard manure in a 3:1:1 ratio (v/v). Diammonium phosphate (DAP) at 2 g $kg^{-1}$ was also added to the potting mixture. At 1–2 leaf stage, seedlings were spray–inoculated with viable sporangia ($1x10^6$ sporangia $ml^{-1}$ of water) of the Patancheru population of the pathogen. Inoculation was done in an inoculation chamber maintained at 20°C and >95% relative humidity (RH). Inoculated seedlings were maintained in this chamber for 16 h to complete the early processes of infection. The pots were then transferred onto greenhouse benches for disease development at temperature not exceeding 30° C. Cultivars NBH 3 and/or 7042(S) and ICML 16 (700651) were used as susceptible and resistant controls, respectively. DM incidence records were taken 20 days after inoculation (Singh and Gopinath 1985; Singh *et al.*, 1993).

**Advanced greenhouse screening.** Two hundred and twenty three accessions, that remained DM free in the preliminary screening, were tested. Each accession was sown in one pot in three replications. NHB 3 was used as susceptible control. Seedlings at coleoptile–to–one–leaf stage were inoculated, maintained, and evaluated as described previously. To maintain uniform seedling stand inoculation, slow or fast growing seedlings (just emerging or those that passed 1–leaf stage) were removed (Singh and Gopinath 1985).

**Field screening.** All the 223 accessions from the advanced screening were tested in field DM nurseries at ICRISAT Patancheru, and at Mysore, India, during the 1992 rainy season (Singh *et al.*, 1993). Each accession was sown in two, 4 m–row–plot at Patancheru, and in a single, 3 m–row–plot at Mysore, in two replications at both the locations. The crop was fertilized with 100 kg DAP $ha^{-1}$ as basal and 100 kg urea $ha^{-1}$ as top–dress. No insecticide or weedicide was applied. DM incidence records were taken twice, 25 days after sowing and at soft dough stage, at both the locations.

**Cross–inoculation.** Five highly DM–susceptible accessions, one each from *P. violaceum*, *P. mollissimum*, *P. purpureum*, *P. pedicellatum*, and *P. polystachyon*, were sown in five pots each, about 35 seed per pot, in the greenhouse. At 1–leaf stage, these accessions were spray–inoculated with sporangia of the Patancheru population of *S. graminicola*. At about 25 days after inoculation, fresh sporangia were produced on each accession, separately collected, and used for inoculation of 48 h old potted seedling of NHB3, as described under preliminary screening. About 100 seedlings in 5 pots, 15–30 seedlings per pot, were inoculated. Non–inoculated NHB3 seedlings were kept as control. DM incidence records were taken 20 days after inoculation.

**Evaluation of *P. schweinfurthii* against three pathogen populations.** Five accessions of *P. schweinfurthii*, that remained DM–free in all the tests, were tested against pathogen populations from Mysore, Rajasthan, and Patancheru. Each accession was sown in five pots, about 30 seed per pot, filled with the potting mixture. DM susceptible genotypes, 7042(S) and NHB 3, were used as controls. Inoculation and evaluation were done as described under advanced greenhouse screening.

**Other diseases.** All the 223 accessions were evaluated for rust (*Puccinia penniseti*) reaction under the natural rust epiphytotic, both at ICRISAT–Patancheru, and Mysore. Five accessions of *P. schweinfurthii* were inoculated with smut [*Tolyposporium pennicellariae* Bref.] (Thakur *et al.*, 1983) and ergot [*Claviceps fusiformis* Loveless] (Thakur *et al.*, 1983) also at ICRISAT Patancheru, during the 1993 rainy season. Five panicles of each accession were inoculated separately with smut and ergot pathogens. NHB 3 was used as control for both the diseases.

## Results and Discussion

**Preliminary greenhouse screening.** DM incidence values ranging from 89 to 100 per cent in the two susceptible controls (NHB 3 and 7042 S) and 19 to 24 per cent in resistant control (700651) are evidences of a severe disease pressure in the test. Under this disease pressure, 223 accessions remained DM free and 14 other showed very high levels (< 5% DM) of resistance (Table 1). The highest frequency of DM free or resistant (< 5% DM) accessions was detected in accessions of *P. pedicellatum* and *P. polystachyon*. Four accessions of *P. schweinfurthii* and 10 of five other species [*P. ramosum* (4), *P. alopecauroides* (1), *P. mezianum* (1), and *P.*

*villosum* (1), *P. mazianum* (1), and *P. hohenacheru* (2)], also remained DM free. *P. violaceum* showed the least frequency (4%) of DM free accessions and also the highest mean (33%) and maximum (94%) DM incidence.

**Advanced greenhouse and field screenings.** All the 223 accessions remained DM free in greenhouse at Patancheru and in field tests at Patancheru and Mysore. DM incidence on susceptible NHB 3 was 92% in the greenhouse test, and 86 per cent and 91 per cent in two field tests at Patancheru and Mysore, respectively.

Interestingly, accessions of *P. violaceum* from Mali showed higher frequency (19% accessions DM free) of DM resistance than those from Niger (<1% accessions DM free) (Table 2). Also, none of the 35 accessions of *P. millissimum* from Niger was DM free as compared to 25 per cent of its accessions from Mali being free. No such differences were observed for other species/country.

**Table 1. Downy mildew (DM) (*Sclerospora graminicola*) reaction[ab] of 539 wild pearl millet (*Pennisetum* spp) accessions in a greenhouse at ICRISAT Center, Patancheru, during the 1992 rainy season**

| Species | No. of accessions | No. of accessions in DM reaction category | | | | | | DM (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1–5 | 6–10 | 11–25 | 26–50 | >50 | Min. | Max. | Mean |
| *P. violaceum* | 273 | 11 | 8 | 28 | 94 | 76 | 56 | 0 | 94 | 33 |
| *P. millissimum* | 41 | 1 | 3 | 6 | 21 | 10 | 0 | 0 | 49 | 20 |
| *P. purpureum* | 12 | 3 | 2 | 2 | 4 | 1 | 0 | 0 | 17 | 10 |
| *P. pedicellatum* | 129 | 127 | 0 | 0 | 1 | 1 | 0 | 0 | 37 | 1 |
| *P. polystachyon* | 69 | 67 | 1 | 0 | 1 | 0 | 0 | 0 | 18 | <1 |
| *P. schweinfurthii* | 5 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 39 | 2 |
| Others[c] | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NHB 3 (Susc. control) | | | | | | | 0 | 99 | 100 | 100 |
| 7042S (Susc. control) | | | | | | | 27[d] | 89 | 92 | 91 |
| 700651 (Res. control) | | | | | | 27[d] | | 19 | 24 | 22 |

[a]Mean of 2 replications (pots) each with 40 to 50 seedlings.
[b]Figure rounded–off to the nearest whole number.
[c]Includes six wild species: *P. ramosum* (4), *P. alopecauroides* (1), *P. mezianum* (1), *P. villosum* (1), *P. hohenacheru* (2), and *P. mazianum* (1).
[d]Number of repetitions.

**Table 2. Relative frequency of occurrence of downy mildew (*Sclerospora graminicola*) resistance (0% DM) in 12 wild pearl millet (*Pennisetum* spp) accessions[a] from 16 countries[b], ICRISAT Center, Patancheru, 1992–1994**

| Species | CAF | CMR | IND | MWI | MLI | MOZ | NER | SLE | SDN | TZA | TGO | UGA | UK | USA | ZMB | ZIM | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. violaceum* | –[c] | – | – | – | 9/48 | – | 1/220 | – | 0/1 | 0/1 | 0/1 | 0/1 | – | 1/3 | – | 0/1 | – |
| *P. millissimum* | – | 0/2 | – | – | 1/4 | – | 0/35 | – | – | – | – | – | – | – | – | – | – |
| *P. purpureum* | 2/3 | 1/5 | 0/1 | – | – | – | – | – | 0/2 | – | – | – | – | – | – | 0/2 | |
| *P. pedicellatum* | – | 5/55 | 56/59 | – | 2/2 | 10/10 | – | – | – | 1/1 | – | – | 0/1 | 0/1 | – | – | 3 |
| *P. polystachyon* | 0/1 | 14/15 | 11/14 | 6/9 | 6/6 | 2/2 | 11/12 | 1/1 | – | 11/13 | – | 1/1 | – | – | 0/3 | – | – |
| *P. schweinfurthii* | – | – | – | – | – | – | – | – | 3/3 | – | – | – | – | 2/2 | – | – | – |
| Others[d] | – | 4/4 | 4/4 | – | – | – | – | – | – | 1/1 | – | – | – | 1/1 | – | – | – |

[a]Number of DM free accessions/total accession.
[b]CAF: Central African Republic, CMR: Cameroon, IND: India, MWI: Malawi, MLI: Mali, MOZ: Mozambique, NER: Niger, SLE: Sierra leone, SDN: Sudan, TZA: Tanzania, TGO: Togo, UGA: Uganda, UK: United Kingdom, USA: United States of America, ZMB: Zambia, ZIM: Zimbabwe.
[c]Not tested.
[d]Includes six species: *P. alopecauroides* (1), *P. hohenacheru* (2), *P. mezianum* (1), *P. mazianum* (1), *P. ramosum* (4), and *P. villosum* (1).

**Table 3. Downy mildew (DM) reaction[a] of five *P. schweinfurthii* accessions after inoculation with three pathogen populations under greenhouse conditions at ICRISAT Center, Patancheru, during the 1993 rainy season**

| Accession | Mysore | | Rajasthan | | Patancheru | |
|---|---|---|---|---|---|---|
| | Plant No. | DM (%) | Plant No. | DM (%) | Plant No. | DM (%) |
| IPW 151 | 18 | 0 | 17 | 0 | 25 | 0 |
| IPW 152[b] | 25 | 0 | 15 | 0 | 15 | 0 |
| IPW 153 | 15 | 0 | 15 | 0 | 5 | 0 |
| IPW 154 | 5 | 0 | 5 | 0 | 15 | 0 |
| IPW 155 | 15 | 0 | 14 | 0 | 22 | 0 |
| 7042(S)(Susceptible) | 12 | 100 | 13 | 100 | 12 | 100 |
| HB3 (Susceptible) | 12 | 75 | 12 | 100 | 12 | 100 |

[a]Mean of five replications each represented by a pot.
[b]This accession developed 38.9% DM.

**Evaluation of *P. schweinfurthii* against three pathogen populations.** None of the five accessions developed DM when inoculated with pathogen populations from Mysore, Patancheru, and Rajasthan (Table 3). However, in a another test with Patancheru population one of the accessions – IPW 152, developed 38.9 per cent DM incidence.

**Cross-inoculation test.** Sporangia from all the five species produced 19–88 per cent DM on pearl millet cultivar, NHB 3 (Table 4). All the five test entries showed clear chlorosis with abundant asexual sporulation. Shape and size of sporangia produced on wild species were similar to those produced on pearl millets and they had normal germination. We did not analyze the infected plants of wild species for the presence of oospores.

**Table 4. Downy mildew (DM) incidence on NHB3 after inoculation with sporangia from five wild species in the greenhouse during 1995 rainy season at ICRISAT Center Patancheru**

| Source of sporangia | Plant number | DM[ab] (%) |
|---|---|---|
| *P. pedicellatum* (IPW 423) | 18 | 56 |
| *P. polystachyon* (IPW 407) | 37 | 19 |
| *P. millissimum* (IPW 248) | 18 | 50 |
| *P. purpureum* (IPW 267) | 21 | 57 |
| *P. violaceum* (IPW 168) | 26 | 88 |
| NHB 3 (Susc. control) | 18 | 98 |

[a]Mean of five replications.
[b]Figures were rounded off to the nearest whole number.

**Other diseases.** No accession developed rust at any location. However, NHB 3, rust susceptible control, showed 40–65 per cent rust severity, with a mean of 40% rust at ICRISAT Patancheru, and 50 per cent at Mysore. This observation, however, needs confirmation.

All the five accessions of *P. schweinfurthii* developed ergot (10–25%) and smut (5–15%) with artificial inoculation. Mean smut and ergot severity on NHB 3 control were 38 per cent and 65 per cent respectively.

Although the levels of DM resistance, and its frequency (percentage of resistant accessions in a given species) varied with the species, the resistance was detected in accessions from all the species. Origin of accessions seems to have little effect on the frequency and/or level of resistance, except in the case of *P. violaceum* and *P. mollissimum* (their Mali accessions had a greater frequency of DM resistance than those from Niger), (Table 2). DM resistance genes in these species are valuable additions to our existing sources of DM resistance from pearl millet (Singh, 1990; Singh *et al.*, 1993). However, we need to determine whether these genes are different from those already available with us.

The methods of transfer of DM resistance genes from wild species to pearl millet will be easier with some species, while difficult with others. For instance, *P. violaceum* crosses with pearl millet in nature and can be used comparatively easily. Also *P. purpureum* (napier grass), which forms the secondary gene pool (Harlan and de Wet, 1971), is a sexual, rhizomatous perennial, and readily crosses with pearl millet. Presence of DM resistance in napier grass, both in terms of resistance levels (83–100%) and its frequency (40% of

the accessions tested showed >95% resistance), coupled with other useful traits like pests resistance, vigorous growth, and good forage yield, is highly encouraging. Its interspecific hybrids with pearl millet can be used as "bridges" for the transfer of useful genes to pearl millet. For the transfer of DM resistance genes from wild species that are cross–incompatible with pearl millet, biotechnological techniques involving transferring of pieces of DNA to pearl millet protoplasts, using vectors or electroporation, can be employed (Hanna, 1987).

Freedom from DM of four of the five accessions of *P. schweinfurthii*, which can be crossed with pearl millet (2), is encouraging. The resistance genes from *P. schweinfurthii* will be of great value in providing a broader genetic base against *S. graminicola* in pearl millet, if these are different from the DM resistance genes already available in pearl millet. Such genes, deployed individually or in combinations in different types of cultivars ($F_1$ hybrids, open pollinated cultivars, etc.), may likely provide durable resistance. Also, all these accessions are rust resistant, it is likely that the resistance to the two diseases may be linked and can be transferred simultaneously. These are the first known sources where 100 per cent resistance to two important pearl millet diseases is present in one source.

Successful cross inoculation of pearl millet with sporangia produced on five wild species (Table 4), indicates that these grasses might be getting infected by the pathogen in nature. As these species are present in almost all the countries where pearl millet is grown, they might be serving as a natural "reservoirs" of oospore and/or sporangial inoculum of *S. graminicola*, contributing to the primary and/or secondary infection of the disease in farmers' fields. They may also be serving as 'breeding ground' for the development of variability in the pathogen. These two factors will have significant implications for the survival of DM resistant cultivars in farmers' fields. Therefore, information on the occurrence of DM on these species in nature, their role in the disease epidemiology in farmers' fields, and on the development of variability in the pathogen, is necessary for the development of strategies of deployment of DM resistance cultivars in different regions/countries (Singh *et al.*, 1987).

The susceptibility of four wild species– *P. pedicellatum*, *P. polystachyon*, *P. millissimum*, and *P. purpureum* to *S. graminicola* from pearl millet; for the first time in this study and of *P. violaceum* in the past (Werder *et al.*, 1989), shows that this pathogen is not as host–specific as it was believed to be. There is a need to test many more accessions of those wild species that have been free in this study to confirm that they are not host of this pathogen.

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