

# Recovery Resistance to Downy Mildew in Pearl Millet

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## ABSTRACT

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The development of symptomless shoots from pearl millet plants that show typical systemic symptoms of downy mildew (DM) disease is reported. This phenomenon, referred to here as "recovery," and the trait as "recovery resistance," was detected in 28 of the 33 pearl millet genotypes studied. These represent a wide range of breeding materials and cultivars. The genotypes showed considerable variability for the frequency of plants with recovery resistance and for the extent of sexual and asexual sporulation of *Sclerospora graminicola* in recovered plants before recovery. The levels of recovery resistance were substantially increased in several genotypes through pedigree selection under high disease pressure in the DM nursery. Injection of growing points of the symptomless shoots of recovered plants with a sporangial suspension, or clipping the stems and shoots of recovered plants growing under high disease pressure, generally did not result in subsequent disease development. Lines produced from recovered plants maintained their resistance at three locations in India. The phenomenon was also observed in Mali and Niger. It is suggested that recovery resistance may be an effective defense mechanism that should be exploited in breeding pearl millet for durable resistance to DM.

Additional keywords: *Pennisetum americanum*

Downy mildew (DM), a systemic disease caused by *Sclerospora graminicola* (Sacc.) Schroet., is the most important disease of pearl millet (*Pennisetum americanum* (L.) Leeke) in India and the Sahelian and sub-Saharan zones of Africa. Host plant resistance, quantified in terms of frequency of disease-free plants, is the most practical method for the control of this disease.

It has generally been accepted that once DM infection reaches the growing point, all leaves and tillers arising from that growing point will become systemically diseased, and heads will be devoid of grain. Recently it was observed at the ICRISAT Center that in certain pearl millet genotypes, plants with systemic symptoms outgrow disease and produce healthy earheads. Although this phenomenon has been reported (1), we are not aware of a host-pathogen system in which it has been studied. The purpose of this paper is to report the widespread occurrence of this phenomenon, referred to here as recovery, in pearl millet plants infected with the DM pathogen, and to present evidence for its potential as an effective mechanism for the control of this disease.

## MATERIALS AND METHODS

### Terminology. The term recovery is

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used here to mean either 1) the production of symptomless leaves in a shoot that already has one or more leaves with systemic DM symptoms, or 2) the production of symptomless shoots on a plant which already has one or more shoots with systemic DM symptoms. In this study, asexual sporulation on the leaf surface was considered an essential sign to confirm systemic DM infection.

**Cultivars.** Thirty-three genotypes of pearl millet of diverse origin were evaluated. These genotypes included selections from germ plasm accessions with stable DM resistance, hybrids and their parents, and varieties released for cultivation (Table 1).

**Inoculation procedures.** Screening was done in the greenhouse and in the field. In the greenhouse, each cultivar was sown in 12- to 18-cm-diameter plastic pots containing a potting mixture of red soil and farmyard manure (2:1, v/v). In the greenhouse, emerged seedlings (5-10 mm aboveground) were inoculated with a suspension of sporangia applied as a drop at the apex of the coleoptile. This inoculation resulted in systemic infection (4). In the field, screening was done in the DM nursery (8) at the ICRISAT Center. This field contains a high level of oospores. It has been used as a DM nursery for the past 10 yr, and each year oospore-bearing debris is incorporated into the soil. Each genotype was sown in a single, 4-m-row plot, unless stated otherwise. Checks of a highly DM-susceptible FI hybrid, NHB 3 (obtained from MAHYCO seeds, Jalna, India), were sown after every 10 test rows.

A syringe inoculation method was used to inoculate symptomless shoots that arose from plants with systemic symptoms. In this method, a suspension of sporangia ( $1 \times 10^5$  spores/ml) was injected into the growing point of each shoot with a hypodermic syringe and 26-gauge needle. S3 progenies of four genotypes, 81 A, 841 A, SDN 503-2, and P 1449, were inoculated in this manner in the greenhouse and in the field.

**Downy mildew incidence and recovery records.** The DM incidence and recovery were recorded 10 days after inoculation and again 20 and 30 days later.

**Classification of recovery.** Plants showing recovery were classified into two types: Type 1 is recovery based on production of symptom-free leaves on the primary shoots after the production of one to several leaves with symptoms of systemic DM (early recovery, early recoverer), and Type 2 is recovery based on production of symptom-free basal shoots on a plant whose primary shoot or primary shoot and one or more secondary shoots exhibited typical symptoms of systemic DM (late recovery, late recoverer).

**Generation advance and selection.** Each genotype was first tested in the greenhouse. The plants that showed recovery and closely resembled the parent type were selfed and their seed was harvested and kept separately. The resultant progenies, grown ear-to-row, were tested in the DM nursery, and the recovered plants were advanced by selfing for three generations (S1 to S3). Major selection criteria were recovery from DM and phenotype like the parent.

Recovered plants of A lines (male-sterile) were maintained by crossing them with recovered plants of the corresponding B line (maintainer line). Details of the number of progenies selected and evaluated in each generation, along with their DM and recovery reactions, are presented in Table 2.

**Greenhouse evaluation.** Four S3 progenies, two of 81 A, and one each of SDN 503-2 and P 1449, were evaluated for recovery resistance. Each entry was sown in nine pots. Generally, two to three recovered plants per pot were maintained. The experimental design was a randomized complete block with three replications (three pots per replication). The seedlings were inoculated at the coleoptile stage (4).

**Agonomic evaluation.** S3 progenies of three genotypes, SDN 503-2, 81 A, and

841 A, were compared with their parent genotypes for tillering, days to 50% bloom, plant height, and earhead length during the 1985 postrainy season in the DM nursery. Seeds of each progeny were sown in a single, 4-m-row plot along with

the parent genotype. Ten randomly selected plants from each progeny were assessed for tillering. Height of main shoots (base to earhead tip) of 10 randomly selected plants and their earhead lengths were measured in each

progeny at the soft dough stage. In the parent genotypes, 20 plants (10 plants per row) were assessed for these traits. Progenies from this trial were also assessed for pathogen sporulation and for the effects that clipping stems and shoots and syringe inoculation had on subsequent development of DM symptoms.

**Statistical analysis.** Significance of the means of the recovered progenies was tested with the means of the parent genotypes (assumed to be true value) using the Student's *t* test, with *df* = (number of progenies - 1).

**Effect of clipping stems and shoots.** Recovered shoots and disease-free shoots produced on recovered plants grown in the DM nursery were clipped just above ground level. This operation, which temporarily retards plant growth, allows expression of latent infection and induces production of tillers that are generally diseased in plants carrying latent infection. Shoots (variable number) of recovered plants in three S3 progenies each of SDN 503-2, 81 A, and 841 A; four S1 progenies of ICMS 7703; and five S1 progenies of WC-C75 were clipped. Downy mildew incidence was recorded 15 days after clipping, and the plants were observed until maturity for subsequent symptom development.

**Assessment of sporulation.** Infected leaves of recovered plants were assessed for sexual and asexual sporulation of the pathogen. Asexual sporulation was assessed in three progenies each of SDN 503-2, P 1449, 81 A, 841 A, and WC-C75, grown in the greenhouse and in the DM nursery. In the greenhouse, potted plants (7-12 days after the first indication of recovery) were irrigated, covered with polythene bags, and maintained at 25-28 °C overnight. On the following morning, infected leaves were detached and examined microscopically for sporangial production. In the field, the crop was irrigated and infected leaves of recovered plants were covered overnight with polythene bags and microscopically examined the next morning. Sporulation was classified as scanty (sporangia few in number, scattered, unevenly

**Table 1.** Downy mildew (DM) incidence and recovery reaction of selected genotypes in the greenhouse

Genotype	Origin	Plants inoculated	Downy mildew incidence (%)	Recovery* (%)
<b>Accessions</b>				
P 8830-2	Zimbabwe	32	9	67
700516	Nigeria	24	17	50
IP 1930-7	India	117	14	44
P 3281-1	Togo	34	20	41
E 298-2-1-8	India	137	18	40
SDN 503-2	Nigeria	86	29	36
EB 83-2-4	India	68	82	25
700512-3	Nigeria	125	20	24
P 43-1	Cameroon	30	30	22
P 1449-2	Senegal	96	33	22
700651	Nigeria	96	9	22
MPP 7147-2-1-8	India	48	42	20
J 1593	India	32	34	18
P 7	Mali	325	4	17
P 310-1	Mali	34	27	11
IP 3646-1	India	29	34	3
7042				
(susceptible check)	Chad	215	84	1
<b>Hybrid parents</b>				
841 A	India	147	25	30
5141 A	India	87	77	28
81 A	India	181	16	27
21 A	USA	139	13	11
834 A	India	297	38	10
111 A	India	155	9	9
23 A	USA	140	92	0
68 A	India	212	72	0
833 A	India	224	5	0
J 104	India	205	97	0 <sup>b</sup>
<b>Advanced breeding material</b>				
ICMH 84814	India	149	31	39
ICMP 84814	India	220	22	2
<b>Cultivars</b>				
BJ 104	India	68	83	25
WC-C75	India	347	15	22
ICMS 7703	India	286	25	8
NHB 3				
(susceptible check)	India	170	88	0

\* Recovery is based on the number of infected plants. The majority of the unrecovered plants died in about 30 days after planting.

<sup>b</sup> Data from the 1982 field screening.

**Table 2.** Downy mildew (DM) incidence and recovery from DM infection in four pearl millet genotypes evaluated in the greenhouse (S0) and in the field DM nursery (S1-S3)

Genotypes	S0 <sup>a</sup>			S1			S2			S3		
	Plants	DM (%)	Recovery (%)	Progenies	Mean DM (%)	Mean recovery (%)	Progenies	Mean DM (%)	Mean recovery (%)	Progenies	Mean DM (%)	Mean recovery (%)
81 A	181	6	27	8	52(41-65) <sup>b</sup>	60(52-80)	10	58(35-70)	74(46-97)	16	56(18-93)	84(52-92)
841 A	147	25	30	5	48(36-75)	57(37-80)	10	68(52-80)	80(41-94)	10	62(26-90)	90(42-96)
SDN 503-2	86	29	36	10	48(25-65)	61(39-90)	7	68(53-81)	76(64-88)	19	66(32-88)	74(33-97)
P 1449	96	33	40	7	48(25-65)	61(39-90)	9	25(8-50)	66(50-90)	...	...	...
NHB 3												
(susceptible check)	170	88	0	0	82	0		79	0		92	0

<sup>a</sup> Genotypes were inoculated using the seedling inoculation method.

<sup>b</sup> Figures in parentheses are DM incidence ranges.

distributed) or profuse (densely produced sporangioophores).

For the detection of oospores, leaves with visible asexual sporulation in the field were tagged and, just before senescence, were detached, cut into small segments, and cleared by boiling for 5 min in 5% NaOH. The cleared segments were mounted in lactophenol and examined microscopically for the presence of oospores. Two to three first-formed leaves from each plant and five to 10 plants from each of the three S3 progenies of SDN 503-2, 841 A, 81 A, and four S1 progenies of WC-C75 were examined.

**Multilocal testing in India.** Five S3 progenies of recovered plants of 81 A, SDN 503-2, and P 1449 were tested for recovery resistance at Mysore, Cuddalore, and at the ICRISAT Center. Seed from each progeny was sown in a single 4-m-row plot at Mysore and Cuddalore and in two 4-m-row plots at the ICRISAT Center. Downy mildew incidence was recorded 32–35 days after sowing.

## RESULTS

**Recovery.** Recovery was observed 10–30 days after inoculation. Before recovery, host infection, colonization, symptom development, and sporulation of the pathogen were observed, but subsequently the plants outgrew the disease. Such plants remained symptomless throughout the remainder of the growth period and produced healthy earheads.

**Preliminary screening.** Genotypes varied markedly (0–67%) for recovery resistance (Table 1). Notable among the recoverers were BJ 104, WC-C75, and ICMS 7703, varieties under commercial cultivation in India; SDN 503-2, 700651, and 700516, lines with stable resistance to DM; and several male-sterile lines including 81 A, L111 A, 841 A, and 834 A, which are being used in hybrid programs in India. NHB 3, once the most popular hybrid in India (now replaced), and several male-sterile lines did not recover.

**Recovery resistance in advanced screening.** Recovery reactions of four genotypes that were advanced for increasing levels of recovery resistance up to S3 are presented in Table 2. Genotypes generally showed an increase in the frequency of plants with recovery type resistance with the advance in generation. High levels of recovery resistance were obtained in S2 and S3 generations of 81 A, 841 A, and SDN 503-2.

**Greenhouse evaluation.** Downy mildew susceptibility and recovery resistance levels of four agronomically superior progenies, one each of SDN 503-2 and P 1449, and two of 81 A obtained in the greenhouse, along with their reactions in the field, are presented in Table 3. All the progenies showed high levels of recovery

in the greenhouse and in the field. Genotypes varied, however, in their recovery pattern. Most of the plants in 81 A-1-4-7, 81 A-4-7-8, and P 1449-1-4-9 showed early recovery, whereas the majority of the plants in SDN 503-2-1-5-17 showed late recovery.

**Agronomic evaluation.** The recovered progenies were similar to their parent genotypes for three of the characters studied (number of productive tillers, height, and earhead length), except for earhead length in SDN 503-2 (Table 4). The recovered progenies, however, were later in flowering than the parent genotype ( $P = 0.05$ ).

**Effect of syringe inoculation.** None of the recovered plants of 81 A, 841 A, and P 1449 developed DM symptoms after syringe inoculation in the field or greenhouse. Symptoms did develop in 7% of the SDN 503-2 plants inoculated in the greenhouse.

**Effect of clipping.** Clipping recovered shoots did not result in development of symptoms on regrowth in four of the five genotypes evaluated. A small percentage (14%) of systemic symptoms did develop on regrowth of WC-C75.

**Effect on sporulation.** Sporulation on

systemically infected leaves of plants that later recovered varied with genotypes (Table 5). The majority of the plants of 81 A and 841 A produced scanty sporulation, whereas profuse sporulation occurred in SDN 503-2 and P 1449. Sporulation was heavy on nonrecovered plants of all the genotypes.

No oospores were detected in infected leaves of recovered plants of 81 A. In infected leaves of recovered plants of 841 A and SDN 503-2, sporulation was sparse and the proportion of oospore-bearing plants was low. Nonrecovered plants that died within 30 days could not be assessed for oospore production. All these cultivars, however, are known from other tests to produce oospores in infected leaf tissue.

**Multilocal testing in India.** Downy mildew pressure was highly variable, being greatest at ICRISAT Center and lowest at Cuddalore. However, recovery occurred in all entries and at locations where disease developed. Recovery was 100% at Cuddalore and it ranged from 50 to 100% at Mysore and 42 to 100% at the ICRISAT Center for the five progenies of the three genotypes tested.

**Table 3.** Plant population, number of downy mildew (DM) infected plants, and plants that recovered (recovery, %) in four S3 progenies of three genotypes evaluated in the greenhouse and in the field DM nursery

Genotype	Plants	Diseased plants	Plants in recovery reaction category <sup>a</sup>		Recovery (%)
			1	2	
81 A-1-4-7 (G) <sup>b</sup>	24	18	10	5	83
81 A-1-4-7 (F)	13	9	4	3	78
81 A-4-7-8 (G)	26	18	7	9	89
81 A-4-7-8 (F)	28	21	10	7	81
SDN 503-2-1-5-17 (G)	17	15	1	12	87
SDN 503-2-1-5-17 (F)	17	13	0	11	85
P 1449-1-4-9 (G)	32	13	10	2	93
7042 (susceptible check)	26	21	0	0	0

<sup>a</sup> Recovery reaction category: 1 = recovery on main shoot after the production of one to several infected leaves; 2 = recovery on basal, secondary shoots of plants which already show DM symptoms on the main shoot, or on the main shoot and one or more basal, secondary shoots.

<sup>b</sup> G = Greenhouse, F = field.

**Table 4.** Days to 50% bloom, number of productive tillers, plant height, and earhead length of recovered progenies; one each of MS 81 A, MS 841 A, and SDN 503-2 along with their parent genotypes<sup>a</sup>

Genotype	Progenies	Days to 50% bloom	Productive tillers	Plant height (cm)	Earhead length (cm)
MS 81 A (P) <sup>b</sup>	...	60	3.3	67	19
MS 81 A (R)	19	61* <sup>c</sup> ± 3.6	3.5NS <sup>c</sup> ± 1.10	68NS ± .41	18NS ± 1.63
MS 841 A (P)	...	50	3.2	87	15
MS 841 A (R)	10	52* ± .36	3.5NS ± 1.80	85NS ± 0.21	15NS ± 0.0
SDN 503-2 (P)	...	64	4.8	92	19
SDN 503-2 (R)	19	68** ± .24	5.1NS ± 1.25	87NS ± 1.19	18** ± 2.27

<sup>a</sup> Parent genotypes were planted in a single 2-row plot.

<sup>b</sup> P = Parent (original) genotypes; R = recovered progenies.

<sup>c</sup> Bulk population.

<sup>d</sup> Significant at \* is  $P = 0.05$ , \*\* is  $P = 0.01$ .

<sup>e</sup> NS = Not significant.

**Table 5.** Asexual and sexual sporulation in downy mildew-infected leaves of recovered plants of five genotypes and susceptible check

Genotype	Asexual sporulation			Sexual sporulation		
	Plants studied <sup>a</sup>	Plants with sporulation <sup>b</sup>		Plants studied	Plants with sporulation	
		Scanty	Profuse		Present	Absent
SDN 503-2 (F) <sup>c</sup>	17	1	16	24	1	23
SDN 503-2 (G)	25	0	25	...	...	...
81 A (F)	41	35	6	25	0	25
81 A (G)	29	23	6	...	...	...
841 A (F)	36	24	12	24	1	23
841 A (G)	32	22	10	...	...	...
P 1449 (G)	16	0	16	...	...	...
NHB 3 (F)						
(susceptible check)	30	0	30	30	30	0
NHB 3 (G)						
(susceptible check)	26	0	26	...	...	...

<sup>a</sup>Plants represent three progenies of each cultivar and five check rows for NHB 3.

<sup>b</sup>Scanty = few sporangia scattered and unevenly distributed; profuse = evenly distributed and densely produced sporangia.

<sup>c</sup>F = Field, G = greenhouse.

<sup>d</sup>Data not taken.

## DISCUSSION

The results show that recovery resistance may provide an effective protection against continued disease development and new infection by *S. graminicola*. However, the mechanism responsible for this phenomenon needs to be determined. Recovered plants of 81 A, 841 A, and P 1449 did not develop disease following syringe inoculation or clipping. This may possibly be due to the production of phytoalexin-like compounds in the recovered plants which either kill the pathogen or inhibit its growth in the host. The disease, however, did develop in some plants after syringe inoculation (in SDN 503-2) or after clipping of stems and shoots (in WC-C75). Though genetic variability within and among genotypes may, in part, explain these phenomena, the precise reasons are to be determined. Recovery from DM in pearl millet after spraying with the systemic fungicide metalaxyl has been reported (5). In recovery following metalaxyl treatment, however, the chemical is fungistatic and recovered plants can again show symptoms, presumably due to a decrease in concentration of the fungicide in the plant.

Type 1 recovery would be the ideal type of recovery to use in a breeding program. In this type, the pathogen develops on a shoot which ultimately produces a healthy earhead. Such plants would also contribute less inoculum to the development of an epidemic than would Type 2 plants.

Genotypes from different geographic areas showed recovery resistance.

Progenies of three genotypes maintained this trait at the three Indian locations where the disease is generally severe. The phenomenon was also observed in farmers' fields in Mali and Niger in 1985 (S. D. Singh, unpublished). These observations suggest that the recovery phenomenon is not location-specific.

The variability for the production of sexual and asexual spores within genotypes may make it possible to isolate lines which support either no sporulation or reduced sporulation. Cultivars carrying recovery resistance with reduced asexual sporulation will curtail exodemic development of DM, particularly in adjacent pearl millet fields sown to susceptible cultivars (6,7). Growing cultivars with recovery resistance could prevent oospore buildup in the soil because they do not support oospore production. They might actually serve as a trap crop which might effectively reduce the oospore population of the soil over time. However, inhibition of oospore production in these genotypes should be confirmed following inoculation with compatible mating types (2).

Cultivars having resistance based on the type of recovery described here might be less likely to break down than those not having this type of resistance. Such cultivars will be uniformly susceptible to infection by oospores of *S. graminicola*. Consequently, all viable and host nonspecific oospores present in soil would be able to cause infection. The pathogen would grow normally inside the host until the initiation of recovery. Since this resistance prevents oospore formation by the pathogen, as was

observed in this study, selection pressure might favor new variants capable of producing oospores. Such variants may not be of much importance if cultivars possessing recovery resistance are grown, since the proportion of infected leaves and, therefore, number of oospores produced would be relatively small. Oospores with host-specific pathogenicity could die out in the absence of the host, as was demonstrated at Durgapura, India, on the cultivar NHB 3 (S. D. Singh & G. Singh, unpublished). Cultivars with recovery resistance, therefore, should be able to withstand DM for a longer period of time than cultivars without this type of resistance. They should not require replacement solely because of their susceptibility to DM, as has happened in the past in India (3). More information on the possible influence of the recovery trait on yield is required, although data presented here (Table 4) suggest that the yield is not reduced.

The recovery phenomenon has not been reported before in pearl millet. Perhaps the initial reading for DM incidence in the field is taken too late to distinguish recovered plants from symptom-free plants. Conversely, DM reaction data obtained on plants grown in the greenhouse are usually based on seedling plants, which are usually destroyed before the recovery phenomenon would become apparent.

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