Genetic Resistance to Pearl Millet Downy Mildew III: Resistance in Photoperiod Sensitive Accessions

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Abstract

Genetic resistance is the most economic and feasible method of control of downy mildew (DM) (*Sclerospora graminicola*) of pearl millet (*Pennisetum glaucum*). Numerous sources of resistance to downy mildew are available is normal flowering millets, however, a systematic search for downy mildew resistance in photoperiod sensitive pearl millet was identified and also with the availability of photoperiod–insensitive line ICML 22, such material can be converted in to day neutral background. To identify genes for DM resistance with diverse origin, we tested 1,030 photoperiod sensitive accessions from 25 countries, in the greenhouse and in field disease nurseries. A total of 50 accessions remained DM free and 300 accessions were in 1–5% DM reaction category against Patancheru pathotype in a preliminary screening under greenhouse conditions. Of the 50 accessions tested, 17 accessions remained completely free from DM infection against Patancheru, and Mysore pathotypes both under greenhouse and field conditions. However, only 11 accessions revealed potential resistance against Patancheru and Mysore pathotypes when tested using the most severe inoculation methods (inject, drop and dip) under greenhouse conditions at Patancheru.

Key words: Genetic resistance, *Sclerospora graminicola*, pearl millet

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 *et al.* 1987, 1993). In western Africa, where improved pearl millet cultivars are not yet widely grown, the disease causes substantial yield losses even in landrace cultivars (S.D. Singh, unpublished).

The disease has been successfully controlled by the use of resistant cultivars which is the most economic and environmentally–appropriate method of control. However, due to highly cultivar–specific nature of the pathogens, resistant cultivars succumb to the disease within 3–5 yr of their large–scale cultivation (Singh and Singh, 1987, Singh 1994). 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, resistance genes from diverse sources/species are needed. Wild relatives of pearl millet, which have been exposed to the pathogen populations for centuries, are one such source. During the last several years, we have tested many photoperiod sensitive lines for their DM reactions. The results are reported in this paper.

Materials and Methods

Germplasm accessions. A total of 1,030 accessions originated from 25 countries (Table 1) were tested for their reaction to DM in the greenhouse and field–disease nurseries from 1990 to 1995. Seeds were obtained from the Genetic Resources Unit of the Genetic Resources and Enhancement Program, ICRISAT.

Preliminary screening for resistance in greenhouse. 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±1°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. DM

susceptible cultivar NHB 3 served as control. DM incidence records were taken 20 days after inoculation (Singh and Gopinath, 1985; Singh *et al.*, 1993).

Advanced screening for resistance in greenhouse and field. Fifty accessions that remained DM free in the preliminary screening were retested under greenhouse conditions and under field DM nurseries during summer and the rainy season 1993 at ICRISAT. In greenhouse test each accession was sown in one pot of 5–inch diameter in three replications. The potting mixture described under preliminary screening was used. HB 3 served as control. Seedlings at coleoptile–to–one–leaf stage were inoculated, maintained, and evaluated as described previously. To maintain uniform seedling stage at inoculation, slow or fast growing seedlings (just emerging or those that passed 1–leaf stage) were removed (Singh and Gopinath, 1985).

Seventeen accessions that remained disease free in the advanced screening were further screened against Patancheru pathotype (pathotype maintained on 7042S) under greenhouse conditions and under field conditions at three Indian locations. Eleven of these accessions were again tested against two Indian pathotypes (Patancheru) and Mysore (pathotype maintained on 852B) using injection, drop and dip methods of inoculation under greenhouse conditions (Singh *et al.*, 1997).

Multilocational screening. All the 50 accessions selected from the preliminary screening, were planted in two 4–m–row–plot at Patancheru in two replications. In a multilocation test, 17 accessions were tested (Singh, *et al.* 1993) during 1993 and 1994 rainy season at ICRISAT–Patancheru, Mysore, and at Aurangabad in India. These accessions were also further screened in field disease nursery during post–rainy season 1993 and 1994 only at ICRISAT. Each accession was sown in two 4–m–row–plot at Patancheru, a single 3–m–row plot at Mysore and Aurangabad, in two replications. The crop was fertilized with 100 kg DAP ha^{-1} as basal and 100 kg urea ha^{-1} as topdress. No insecticide or weedicide was applied. DM incidence (%) records were taken twice, 25 days after sowing and at soft dough stage, at all the three locations.

Results and Discussion

Preliminary screening in greenhouse. Of the 1,030 accessions, 50 were free, 301 showed very high levels resistance (≤ 5% DM incidence). The majority of the accessions in these two groups are from Burkina Faso. About 30% of the accessions showed more than 10% DM (Table 1). Highest level of DM occurred in accessions from Tanzania.

Table 1. Preliminary evaluation of 1,030 pearl millet photoperiod sensitive accessions for downy mildew resistance (%) under greenhouse conditions at ICRISAT

Origin	Accessions tested	Downy mildew incidence (%)					
		0	1–5	6–10	>10	Min	Max
Benin	18	1	4	4	9	0	33
Botswana	2	0	0	1	1	8	42
Burkina Faso	347	30	144	102	71	0	30
Central African Republic	60	5	17	26	12	0	32
Cameroon	235	4	54	74	103	0	50
Gambia	1	0	0	1	0	*	10
India	91	1	16	18	56	0	63
Kenya	5	0	0	2	3	*	–
Malawi	2	1	0	0	1	0	18
Mali	23	2	2	6	13	0	50
Mozambique	20	0	4	1	15	3	49
Namibia	13	0	8	4	1	3	38
Niger	19	0	5	11	3	2	25
Nigeria	8	0	0	2	6	8	23
Senegal	92	4	32	26	30	0	73
South Africa	1	0	0	0	1	*	29
Sudan	20	1	2	8	9	0	41
Tanzania	13	0	0	2	11	8	90
Togo	18	1	8	6	3	0	23
USA	2	0	0	1	1	6	15
Uganda	6	0	0	1	5	7	17
Yemen	2	0	0	0	2	19	28
Zambia	9	0	2	6	1	5	11
Zimbabwe	21	0	3	4	14	3	35
Total	1,030	50	301	307	372		

Advanced screening for resistance in greenhouse and field. Most of the 50 accessions from the preliminary screening remained DM free under greenhouse and field DM nurseries at ICRISAT (Table 2). However, for various reasons only 17 accessions were further tested during the 1993 post rainy season and the 1994 rainy season at ICRISAT, and during the 1994 rainy season at Aurangabad, Mysore (Table 3).

In the most severe inoculation methods, spray, inject, drop and dip inoculations under greenhouse conditions (Singh *et al.*, 1997), only 11 accessions showed resistance against Patancheru and Mysore pathotypes. Seven of these accessions like IP 5875, IP 5952, IP 8510, IP 10429, IP 10434, IP 13019 and IP 13042, showed complete resistance to both the

Table 2. Evaluation of 50 pearl millet accessions under greenhouse and field conditions against Patancheru pathotype at ICRISAT

IP Numbers	Identity	Origin	DM Incidence (%)		
			GH	Field[1]	Field[2]
5875	P 1478/SL 175	Senegal	0	0	0
5938	P 1545/SL 347	Senegal	0	0	0
5952	P 1560/SL 379	Senegal	0	0	0
6026	P 1646/SL 639	Senegal	0	0	0
8510	P 1598/SL 520	Senegal	0	0	0
8743	25 KS–2	Sudan	0	0	6.3
9300	P 3333	Togo	*	0	0
10420	P 3821	Benin	0	2	0
10429	P 3861	Benin	0	0	0
10434	P 3889	Benin	0	0	0
10439	P 3932	Benin	0	3	0
11347	CVP 279	Burkina Faso	0	0	0
11350	CVP 291	Burkina Faso	0	0	0
11547	P 6009	Burkina Faso	0	0	0
11585	P 6054	HVO	0	0	0
12448	Single Plant 18	India	0	0	0
12863	CVP 409–1	Burkina Faso	0	0	0
12881	13–1	Burkina Faso	0	0	0
12900	P 60–1	Cameroon	0	0	7.1
12996	P 374–1	Mali	0	0	0
13019	P 615–1	Mali	0	0	0
13042	P 5615–1–1	Mali	0	0	0
14248	AD 225	Cameroon	0	0	0
14295	AD 341	Cameroon	0	0	0
14875	AD 1317	Cameroon	0	0	0
15523	129	Burkina Faso	0	0	0
15525	131	Burkina Faso	0	0	0
15560	166	Burkina Faso	0	9	0
15567	173	Burkina Faso	0	0	0
15574	180	Burkina Faso	0	0	0
15611	219	Burkina Faso	0	0	0
17212	243	Burkina Faso	0	0	0
17240	257–1	Burkina Faso	0	0	0
17300	290	Burkina Faso	0	0	0
17302	291	Burkina Faso	0	0	0
17304	292	Burkina Faso	0	0	0
17308	294	Burkina Faso	0	0	0

Table 2 Continued....

IP Numbers	Identity	Origin	DM Incidence (%) GH	Field[1]	Field[2]
17311	296	Burkina Faso	0	0	0
17319	301	Burkina Faso	0	0	0
17323	303	Burkina Faso	0	0	0
17324	303–1	Burkina Faso	0	0	0
17326	304–1	Burkina Faso	0	0	0
17328	305–1	Burkina Faso	0	0	0
17333	308	Burkina Faso	0	0	0
17435	AK 379	Central African Republic	0	0	0
17443	AK 410	Central African Republic	0	0	0
17448	AK 418	Central African Republic	0	0	0
17456	AK 433	Central African Republic	0	0	0
17459	AK 436	Central African Republic	0	0	0
17492	ARD 1	Togo	0	0	0
Controls					
700651 (R)			22	9	28
7042 (Susc.)			100	82	97
HB 3 (Susc.)			98	80	97

Mean of two repetitions each with two replications. * = Accessions those were free from DM infection in the preliminary screening under greenhouse conditions against Patancheru pathotype. Field [1] = Summer DM nursery 1993 and field [2] = rainy season DM nursery 1994.

Table 3. Performance of 17 pearl millet accessions in an advanced screening for downy mildew resistance in greenhouse conditions at ICRISAT, and in field DM–nurseries at Aurangabad (Abd), Mysore (Mys), and Patancheru (Ptn)

IP numbers	Identity	Origin	DM incidence (%) GH test Ptn	Field tests Ptn[1]	Ptn[2]	Abd[3]	Mys[3]	Ptn[3]
5875	P 1478/SL 175	Senegal	0	0	0	6	0	0
5938	P 1545/SL 347	Senegal	0	0	0	0	0	6
5952	P 1560/SL 379	Senegal	0	0	0	0	*	0
6026	P1646/SL 639	Senegal	0	0	0	0	0	5
8510	P1598/SL 520	Senegal	0	0	0	3	0	1
8743	25 KS –2	Sudan	0	0	0	3	0	4
9300	P 3333	Togo	0	*	0	0	0	2
10420	P 3821	Benin	0	*	0	0	*	0
10429	P 3861	Benin	0	0	0	0	0	0
10434	P 3889	Benin	0	0	0	0	0	3
10439	P 3932	Benin	0	*	0	0	0	2
11585	P 6054	HVO	0	*	0	0	0	2
12900	P 60–1	Cameroon	0	0	0	0	*	14

Table 3 Continued....

			DM incidence (%)					
			GH test	Field tests				
IP numbers	Identity	Origin	Ptn	Ptn[1]	Ptn[2]	Abd[3]	Mys[3]	Ptn[3]
12996	P 374–1	Mali	0	*	0	0	0	2
13019	P615–1	Mali	0	0	0	0	0	23
13042	P 5615–1–1	Mali	0	*	0	0	0	2
14248	AD 225	Cameroon	0	*	0	0	0	0
Controls								
HB 3 (S)			98	93	100	61	77	100
7042 (S)			100	96	99	86	100	96
700651 (R)			12	7	10	9	0	8

Mean of two repetitions each with two replications. [1] = Rainy season DM nursery 1993, [2] = Post rainy season DM nursery 1993, [3] = Rainy season DM nursery 1994, * = Data not available.

Table 4. Evaluation of 11 promising accessions for downy mildew (DM) resistance against Patancheru (Ptn) and Mysore (Mys) pathotypes under greenhouse conditions using diverse inoculation methods

	DM incidence (%) in greenhouse conditions							
	Spray		Inject		Drop		Dip	
IP numbers	Ptn	Mys	Ptn	Mys	Ptn	Mys	Ptn	Mys
5875	0	0	0	0	0	0	0	0
5938	6	0	0	0	0	0	0	8
5952	0	0	0	0	0	0	0	0
6026	0	0	0	0	10	0	0	0
8510	0	0	0	0	0	0	0	0
10429	0	0	0	0	0	0	0	0
10434	0	0	0	0	0	0	0	0
12900	0	5	0	0	0	4	0	24
13019	0	0	0	0	0	0	0	0
13042	0	0	0	0	0	0	0	0
Controls								
HB 3	100	97	78	75	80	81	100	81
7042(S)	91	91	*	*	74	71	100	95
700651(R)	29	46	40	17	6	35	33	*

Mean of two repetitions each with two replications. * = Data not available.

pathotypes and in the four inoculation methods. IP 12900 and IP 5938 developed DM with Mysore pathotype and IP 6026 and IP 8743 developed DM with both the populations. Other accessions were susceptible. A resistant control (700651) showed DM incidence ranging from 29–46 in spray, 17 –40 in inject, 6 –35 in drop and 33 % in dip inoculation (Table 4).

Although resistance sources are available in normal flowering plants, wild genotypes and genetic stocks, an attempt to evaluate sources of resistance in photoperiod sensitive accessions from at least 25 countries yielded few sources of resistance. Previously we have reported various levels of resistance, and frequency levels in at least 12 wild species of pearl millet originating from 17

countries (Singh and Navi 2000). Interestingly, origin of accessions seems to have little effect on the level of resistance (Table 1). Resistance genes available in photoperiod sensitive accessions are valuable additions to our existing resistance sources from pearl millet (Singh, 1990; Singh, 1994; Singh *et al.*, 1993; Singh and Navi 2000).

Transfer of genes from photoperiod sensitive species in to normal flowering could be possible using biotechnological techniques involving transferring of pieces of DNA to pearl millet protoplasts using vectors or electrophoration.

In case of wild species, freedom from DM of all the resistant accessions of *P. schweinfurthii*, which can be crossed with pearl millet (Hanna and Dujardin, 1985), is encouraging. Such attempts could be possible in photoperiod sensitive accessions giving dark treatment under controlled conditions. In any case, resistance genes will be of great value in providing a broader genetic base against *S. graminicola*, in pearl millet. If these are different from the resistance genes already available, such genes deployed individually or in combinations, in different types of cultivars (F1 hybrids, open pollinated cultivars etc.) may likely provide durable resistance.

There is a scope to test for rust resistance if these accessions are also 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% resistance to pearl millet downy mildew is explored.

Since, some of these photoperiod accessions showed extremely high level of resistance to three population of the pathogen, these are likely to possess broad spectrum resistance. Therefore, these are valuable addition to our existing sources of resistance. Since photoperiod types remain in the field for longer period of time, these are exposed to the pathogens for a much longer period. It is then possible that these pearl millet may probably have resistance genes different from those present in cultivated types. There is a need to determine the differences between the genes present in different kind of material. If found different than the gene from these material will be more useful than those present in wild type because of the cross compatibility with the cultivated types. Such genes deployed individually or in combination in different types of cultivars (F1 hybrid, open pollinated cultivar etc) may likely to provide durable resistance to this disease.

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