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Genetic Variability for Downy Mildew Disease Incidence against Virulent Downy Mildew Isolates in Mapping Population of Pearl Millet

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A B S T R A C T

Downy mildew caused by *Sclerospora graminicola* (Sacc.) Shroet. is a major biotic constraint to pearl millet production in the semi-arid tropics. The pathogen is heterothallic and frequent recombination leads to evolution of new virulent populations. Identification of resistance to new virulent isolates is a prerequisite for resistance breeding. In the present investigation, 295 RILs along with five control entries were screened against three Indian populations of *Sclerospora graminicola* under greenhouse conditions. Among the RILs under study, 19 RILS were found to be highly susceptible (>80 % DMI) in screening against Indigenous pathogen isolates from Gujarat (Sg445) and Haryana (Sg519), 17 RILS were found to be highly susceptible (>80 % DMI) in screening against Indigenous pathogen isolates from Gujarat (Sg445) and Rajasthan (Sg526), and there are no RILs found to be susceptible against three isolates. Among all the mapping populations, 13 RILs were resistant (<10% DMI) to three test isolates. Remaining RILs exhibited different levels of DM incidence to pathogen - isolates.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), is an important cereal crop. It is especially important as a staple food grain and source of feed and fodder for livestock, in hot, dry marginal agricultural production environments of Africa and South Asia that are home to millions of the world's poorest farmers (Rajaram *et al.,* 2013).

Pearl millet, with 8-19% seed protein content and 56-65% carbohydrates is nutritionally superior to rice, sorghum and maize. Pearl millet downy mildew is the most destructive disease that is responsible for substantial economic losses. The estimated annual grain yield loss due to downy mildew is approximately 20-40% (Thakur *et al.*, 2008). The disease is more severe on genetically homogeneous single-cross pearl millet hybrids, which are grown on about 60% of the total 9.5 million ha in India, than on heterogeneous open-pollinated varieties

(Thakur *et al.*, 2006). During the 1970s- 80s several downy mildew epidemics occurred in India resulting in considerable yield losses and withdrawal of several hybrids from cultivation (Singh *et al.*, 1987; Thakur, 1999). Currently, over 70 different hybrids are being grown in India (Thakur *et al.*, 2006) and during our recent on-farm survey, some of them have shown downy mildew incidence up to 100%. The on-farm downy mildew surveys in the major pearl millet growing states of India have revealed that several commercial F_1 hybrids being grown in different states become susceptible to the disease within 3-5 years (Thakur *et al.*, 2003; Rao *et al.*, 2005; Thakur *et al.*, 2006). Existence of mating types and their frequency greatly contribute towards the development of new recombinants in the pathogen populations (Pushpavathi *et al.*, 2006a). Evolution of host-specific virulence in pearl millet downy mildew is well documented (Thakur *et al.*, 1992; Sastry *et al.*, 2001; Pushpavathi *et al.*, 2006b). Since management of pearl millet downy mildew largely depends upon host plant resistance, evolution of new virulence(s) in the pathogen population and resistance effective against new pathotypes need to be periodically monitored. Effective and economic control of this disease can be achieved by growing disease resistant varieties and hybrids. So, disease resistance is a major concern in pearl millet improvement programme. In order to develop disease resistant cultivars, it is important to identify the germplasm lines which can be used as parents for the development of mapping populations which invariably useful in identification of QTLs and candidate genes governing resistance to the DM.

Materials and Methods

The present experiment was carried out with mapping population of 295 RILs develped by crossing of 81B-P13 and AIMP 92901-deriv-P03 at ICRISAT and two control entries *viz.*, 7042(S) and IP18292. They were evaluated against three isolates of downy mildew pathogen namely Sg445 (Gujarat), Sg519 (Haryana) and Sg526 (Rajasthan) under greenhouse conditions during *Kharif* 2013, at ICRISAT, Patancheru. Seed of these accessions were obtained from ICRISAT Genetic Resources.

A wide diversity of populations of this pathogen has been identified from India and samples of these are being maintained at ICRISAT, Patancheru (Thakur and Rao, 1993). The pathogen populations are maintained on plants of highly downy mildew susceptible pearl millet genotypes 7042(S) and F_1 hybrid NHB 3, both of which show >80% infection under heavy inoculum pressure.

The infected plants are grown in sterilized soil in covered pots in a greenhouse room maintained at slightly above atmospheric pressure to prevent the entry of air-borne spores. Seedlings were inoculated at the two or three leaf stage by spray application of a freshly prepared, chilled suspension of pathogen population. The pots were then covered with polythene bags and incubated at 20°C to promote infection. After 12 hours, the bags were removed and the pots of seedlings infected with pathogen population were maintained at 20-25°C in plexiglass-covers on benches in the greenhouse.

The pot-grown seedlings were Inoculated from its maintainer host with the sporangial suspension of the isolate were incubated at 20°C for 16 h in the dark and then transferred to a greenhouse under misting for 4-5 days. The seedlings were grown for 25-30 days at 25 ±2°C under proper care of watering and fertilization to produce good infected foliage, which sporulated profusely and provided a good amount of sporangial inoculum needed for mass inoculation (Thakur *et al.*, 2011).

The experimental material were sown at uniform depth in the holes (single seed per hole) to achieve uniform emergence of seedlings. Seedlings were grown in 12 cm diameter plastic pots, filled with a potting mixture of three quarters consisting of soil, sand and farmyard manure in a 3:2:2 by volume and watered them. In the pots uniform holes (1 cm) were made in saturated soil in the pots using a dibbler stamp (This equipment, which facilitates equidistant sowing of seeds at equal depth in a pot thus reducing variability in emergence time due to sowing depth and distance between seedlings, is a new development). The seeds were covered with a 1-cm layer of potting mixture, irrigated properly and maintained these pots in the greenhouse at 35°C till seedling emergence.

The seedlings were counted at the coleoptile to first-leaf stage (3 days after sowing) in each pot and recorded the number on the plastic label (Seedlings in each pot are counted and recorded on the plastic label before inoculation to discount any seedlings emerging after inoculation). The above pots were transferred into the inoculation room on metallic shelves and organized them in rows. Seedlings were inoculated with the sporangial suspension using pneumatic atomizer till run-off ensuring that every seedling has received uniform inoculum. Inoculated seedlings were covered with a polyethylene sheet immediately to provide high humidity required for infection and incubated in the dark at 20°C for 16-20 h.

The inoculated seedlings were shifted to greenhouse benches at 25 ± 2°C with misting to provide high humidity (>95% RH) and leaf wetness for disease development for the next 14 days. Infected seedlings in each pot were counted and recorded the number on the same plastic label in the pot on which total seedling counts were recorded before inoculation. The same inoculation procedures were repeated three times [time replications in Completely Randomized Block Designs for each of the three pathogen populations]. Mean downy mildew disease incidence percentage will be calculated for each genotype. Symptoms as distinct chlorosis on leaves and stunted growth of seedlings were recorded. The disease incidence was scored as: highly susceptible - DMI>80%, **s**usceptible 50%-80% DMI, moderately susceptible 25% - 50%DMI, moderately resistant 10% - 25%DMI and resistant <10% DMI.

Results and Discussion

Among the 295 RILs under study, 19 lines i.e.5, 19,40, 50,60, 62,73,76,78, 91, 122, 179, 185,186, 205, 225, 238, 274 and 289 were found to be highly susceptible (>80 % DMI) in DM screening against the pathogen isolates from Gujarat (Sg445) and Haryana (Sg519) (Table 1).

Seventeen lines exhibited highly susceptible (>80 % DMI) in DM screening against the pathogen isolates from Gujarat (Sg445) and Rajasthan (Sg526) (Table 1), numbered those are 68, 103, 107, 120, 134, 136, 145, 146, 155, 164, 166, 174, 203, 233, 248, 278 and 290 among 295 RILs.

Among all the 295 Recombinant Inbred Lines, there are no RILs found to be susceptible against all the three pathogen isolates.

Only thirteen lines shown resistance against all the three isolates, those are 22, 42, 53, 61, 69, 74, 75, 189, 228, 230, 237, 281 and 291.

Different populations exhibited different levels of DM incidence with three isolates of the pathogen *Sclerospora graminicola* under investigation.

Summary revealed that the pathogen population of *S. graminicola* from Gujarat (Sg445) caused the greatest disease incidence (62.64%) followed by the pathogen populations from Haryana (Sg519) and Rajasthan (Sg526). A very high heritability estimates for DM1 was observed for screens against Sg526, followed by Sg519 and Sg445.

Table.1 The Mean downy mildew incidence percentage (DMI %) data from 295 RILs, parents and control entries based on cross 81B-P13 and AIMP 92901-deriv-P03 against three isolates of pathogen *Sclerospora graminicola*

S. No.	DMI%		
	Sg445	Sg519	Sg526
1	96.00	0.00	28.15
2	30.83	5.60	0.00
3	85.50	11.00	46.10
4	49.13	27.67	5.95
5	86.60	80.23	1.65
6	70.20	91.43	19.10
7	88.20	0.00	0.00
8	78.07	6.60	6.50
9	92.87	4.87	55.50
10	25.87	0.00	0.00
11	46.67	23.57	10.25
12	94.73	6.60	51.00
13	5.40	35.97	5.45
14	85.90	44.20	58.55
15	95.23	0.00	57.30
16	19.40	0.00	2.65
17	17.77	14.07	7.05
18	95.70	66.23	27.70
19	88.83	93.40	2.40
20	73.80	0.00	17.45
21	8.97	42.70	8.70
22	9.39	1.50	2.65
23	95.27	11.53	23.30
24	85.33	15.33	78.90
25	94.70	9.70	75.10
26	24.67	0.00	24.40
27	86.63	9.67	47.05
28	59.43	0.00	17.05
29	44.57	6.53	16.05
30	3.37	11.97	1.45
31	19.10	11.03	4.60

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
32	93.20	0.00	33.90
33	53.03	0.00	4.45
34	76.57	5.97	18.80
35	94.37	0.00	55.20
36	88.87	15.13	33.30
37	98.40	51.27	0.00
38	26.80	6.90	11.45
39	65.10	0.00	5.15
40	97.63	100.00	15.00
41	96.17	2.77	0.00
42	6.00	0.00	1.85
43	35.73	50.00	0.00
44	92.60	7.13	38.70
45	57.83	12.60	1.40
46	60.80	86.63	28.60
47	97.93	8.93	30.85
48	60.87	13.20	22.00
49	39.50	24.27	0.00
50	98.03	100.00	6.75
51	24.10	25.17	0.00
52	17.67	8.10	2.25
53	5.30	1.13	0.00
54	87.83	60.23	24.75
55	92.47	50.33	13.70
56	55.17	66.67	6.25
57	13.40	26.50	2.00
58	48.43	90.90	15.25
59	43.50	0.00	1.45
60	85.23	100.00	3.75
61	0.00	0.00	1.45
62	97.93	98.23	21.55
63	83.90	14.47	34.50
64	87.10	1.13	21.75
65	12.73	0.00	3.00
66	82.77	12.27	3.35
67	82.60	0.00	0.00
68	96.30	1.20	98.65
69	0.00	1.20	0.00
70	66.13	2.57	16.05

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
71	9.37	14.63	8.30
72	87.73	75.63	33.30
73	92.47	91.67	17.90
74	3.20	1.50	2.25
75	3.27	0.00	7.40
76	97.53	98.67	7.95
77	76.73	13.40	89.90
78	95.47	100.00	8.60
79	44.47	31.20	5.95
80	81.60	13.07	17.00
81	63.47	3.03	8.85
82	14.70	0.00	89.55
83	66.67	25.23	42.90
84	28.60	53.97	2.80
85	67.57	97.90	6.25
86	3.83	17.40	3.75
87	66.63	4.57	19.40
88	37.20	0.00	2.25
89	75.07	20.83	9.35
90	91.33	1.40	63.95
91	100.00	98.23	9.25
92	100.00	43.80	77.10
93	45.83	31.13	12.10
94	60.47	1.07	31.40
95	24.30	0.00	19.95
96	75.63	13.63	45.65
97	1.60	27.73	1.70
98	87.90	1.07	8.40
99	16.90	1.20	10.85
100	74.43	2.47	11.05
101	78.33	47.50	9.45
102	96.13	0.00	9.75
103	93.07	5.40	100.00
104	34.73	0.00	3.35
105	65.90	0.00	9.25
106	25.00	10.07	100.00
107	95.70	10.73	100.00
108	71.10	25.57	59.80
109	28.33	0.00	33.30

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
110	32.73	0.00	45.50
111	64.87	8.63	3.55
112	84.50	4.47	3.15
113	65.80	22.40	31.95
114	98.13	12.63	9.70
115	64.37	88.93	6.40
116	90.40	24.67	40.50
117	37.80	14.03	5.55
118	72.60	53.73	63.40
119	71.93	29.43	2.80
120	97.57	2.23	87.45
121	31.80	69.07	0.00
122	94.90	100.00	11.90
123	34.90	23.40	1.45
124	97.83	1.07	3.05
125	33.30	0.00	22.45
126	91.83	17.77	14.35
127	100.00	17.80	7.15
128	40.63	80.53	0.00
129	82.43	18.67	7.55
130	86.23	20.13	17.10
131	91.17	26.33	23.95
132	45.70	22.33	1.65
133	79.53	0.00	0.00
134	89.83	0.00	86.55
135	100.00	46.67	67.95
136	91.87	27.53	90.90
137	33.80	21.53	2.50
138	83.83	0.00	54.25
139	28.20	0.00	13.40
140	28.93	42.90	21.85
141	12.43	0.00	1.85
142	74.17	73.63	59.40
143	86.30	1.67	10.30
144	23.77	22.77	1.65
145	81.43	64.73	87.35
146	98.03	10.87	83.20
147	75.10	100.00	5.65
148	74.81	71.45	23.60

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
149	92.90	0.00	25.50
150	82.93	26.83	14.75
151	34.80	2.43	9.40
152	96.87	6.07	24.45
153	97.63	10.13	34.85
154	81.93	0.00	5.80
155	95.90	3.07	93.35
156	9.90	12.63	0.00
157	16.97	0.00	0.00
158	13.83	0.00	0.00
159	16.27	2.37	7.50
160	21.07	10.17	0.00
161	36.63	11.03	0.00
162	33.27	9.10	0.00
163	75.33	11.97	5.30
164	95.77	4.20	88.95
165	75.07	39.43	30.95
166	94.43	2.37	87.50
167	53.90	96.43	33.85
168	89.93	2.10	47.70
169	89.00	61.70	6.30
170	25.70	4.10	23.00
171	100.00	0.00	0.00
172	95.47	15.40	4.05
173	74.83	9.43	28.25
174	100.00	8.57	98.10
175	15.87	0.00	0.00
176	58.73	1.67	0.00
177	45.77	28.77	2.85
178	48.50	6.03	1.30
179	98.67	98.57	43.75
180	97.43	6.80	24.50
181	84.97	0.00	0.00
182	70.20	8.97	4.40
183	92.40	7.83	62.75
184	44.20	14.03	7.00
185	95.23	94.87	25.65
186	96.80	96.63	0.00
187	66.87	64.63	0.00

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
188	57.93	77.27	0.00
189	0.00	0.00	0.00
190	93.50	1.67	8.35
191	39.13	15.47	5.25
192	64.07	9.10	11.50
193	12.03	64.03	4.95
194	50.87	72.23	22.45
195	100.00	65.10	0.00
196	62.73	0.00	11.25
197	98.23	45.83	57.40
198	86.53	0.00	21.30
199	88.03	77.23	0.00
200	60.13	23.50	25.45
201	71.10	21.90	11.00
202	34.17	83.00	5.15
203	92.07	0.00	91.30
204	62.03	25.27	51.90
205	86.67	100.00	51.65
206	10.90	33.47	4.90
207	3.17	57.07	0.00
208	95.83	0.00	42.95
209	17.07	0.00	0.00
210	84.90	62.23	0.00
211	71.00	0.00	0.00
212	28.27	1.27	1.90
213	54.77	0.00	95.85
214	55.33	4.43	14.25
215	39.27	0.00	4.75
216	25.73	1.87	0.00
217	87.60	2.10	1.50
218	95.27	11.67	16.45
219	4.30	12.17	0.00
220	33.30	0.00	8.15
221	86.77	63.57	27.20
222	99.93	42.20	22.50
223	97.43	9.50	12.90
224	5.33	11.47	0.00
225	100.00	81.53	15.00
226	85.20	31.77	30.80

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
227	86.30	51.33	38.65
228	1.60	3.37	4.05
229	74.23	2.93	93.65
230	6.40	0.00	8.70
231	25.10	2.17	0.00
232	26.23	15.60	4.95
233	97.43	45.57	100.00
234	72.07	100.00	45.30
235	95.13	11.33	7.40
236	84.53	8.97	44.25
237	7.83	0.00	0.00
238	100.00	100.00	30.90
239	100.00	1.60	63.95
240	85.37	100.00	16.20
241	70.27	0.00	24.50
242	91.30	12.77	10.55
243	98.60	4.20	76.40
244	98.13	3.03	24.40
245	29.97	12.80	9.35
246	92.23	0.00	5.15
247	92.07	1.27	21.15
248	98.73	28.03	98.20
249	14.73	2.57	10.10
250	61.70	10.33	34.05
251	48.03	69.30	7.70
252	100.00	3.33	70.85
253	33.87	0.00	8.40
254	82.57	91.40	6.80
255	55.47	32.80	51.10
256	75.63	6.33	24.40
257	95.70	10.57	39.25
258	13.00	39.73	10.90
259	92.03	7.20	47.90
260	47.37	4.83	2.65
261	94.93	0.00	10.80
262	24.10	55.23	52.25
263	93.87	0.00	55.25
264	0.00	0.00	13.70
265	55.03	0.00	23.90

S. No.	DMI%		
	Sg445	**Sg519**	**Sg526**
266	14.00	0.00	14.30
267	69.67	0.00	96.75
268	95.30	2.30	41.75
269	100.00	64.07	51.30
270	73.67	70.67	35.70
271	53.80	55.63	2.50
272	97.93	24.53	3.15
273	91.40	98.23	5.60
274	100.00	100.00	30.00
275	98.77	37.70	14.25
276	15.40	0.00	0.00
277	2.43	13.07	2.65
278	100.00	24.07	87.80
279	65.20	25.57	13.80
280	72.63	26.07	0.00
281	5.87	0.00	0.00
282	23.90	1.87	0.00
283	3.70	5.17	34.90
284	100.00	14.40	68.35
285	89.67	14.17	18.35
286	70.67	93.43	30.50
287	74.67	31.70	54.40
288	74.17	0.00	8.35
289	98.23	93.77	25.65
290	96.73	36.63	100.00
291	5.13	0.00	2.65
292	62.33	66.40	18.35
293	22.80	3.47	2.95
294	68.98	14.27	13.80
295	74.03	5.67	20.60
P1	100	100	91.15
P2	3.0	3.2	2.0
7042(S)	100	100	94.4
IP18292	64.46	100	71.9
Mean	62.64	26.19	24.38
SEm+/-	4.70	2.82	1.68
SEd+/-	6.66	3.99	2.39
CV (%)	13.023	18.68	9.81
Heritability (broad sense)	0.93	0.97	0.99

Host plant resistance is the most economic and efficient strategy for the management of downy mildew of pearl millet. Effective resistance breeding programmes require close monitoring of virulence change in the pathogen and identification of new resistance sources to the new virulent strains. Virulence change in *S. graminicola* populations is monitored through a collaborative pearl millet downy mildew nursery, on-farm surveys for downy mildew incidence and by characterizing pathogen isolates collected from highly susceptible cultivars in the farmers' fields on a set of putative differential hosts (Sivaramakrishnan *et al.,* 2003; Thakur *et al.,* 2004).

Breeding crop varieties with durable resistance to diseases is made difficult by the variability in the pathogen populations (Christ *et al.,* 1987; Leonard, 1977). Genetic resistance in a cultivar at one location may not function at another location because of the differences in virulence in the pathogen populations (Flor, 1971; Kulkarni and Chopra, 1982; Vanderplank, 1984). Differential host varieties are useful in the analysis of pathogen variability at various locations on the basis of clearly visible resistant and susceptible reactions (Vanderplank, 1984; Wolfe and Knott, 1982). According to Flors (Flor, 1971) gene-for-gene hypothesis, a series of inbred lines with distinct resistance genes will differentiate discrete races of the pathogen. Theoretically, pathogen populations are composed of numerous strains, each of which exists at certain frequency that describes the probability of encounter (Gale, 1987).

Genetic management of downy mildew in pearl millet could be strategically planned on a regular basis, because of a highly dynamic *S. graminicola*–pearl millet system. The International Pearl Millet Downy Mildew Nursery (IPMDMN) conducted to test the stability of resistance also provided evidence of variable pathogen populations in countries in Africa and India (Singh, 1995; Singh *et al.,* 1993).

The control entries 7042(S) showed 94.4-100% DMI and IP 18292 was found to possess 64.46 - 100% DMI across all Indian isolates of pathogen.

The results of this study indicate the importance of identifying Downy Mildew Incidence for an effective downy mildew resistance breeding in pearl millet.

The results of this study can be used to mapping of DMR QTLs and this study also indicating the variability in the virulence level of pathogen isolates for the same genotypes, which aggravating the necessity of host-pahotype specific resistance breeding.

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