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Genetic Variability for Downy Mildew Disease Incidence in Mapping Population Parents of Pearl Millet

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ABSTRACT

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, forty parents along with five control entries were screened against three Indian populations of Sclerospora graminicola under greenhouse conditions. Among the parental lines under study, ICMP 85410-P7, LGD-1-B-10, Tift 23DB-P1-P5, H77/833-2-P5, H77/833-2, Tift 238D1, ICMB 89111-P6, 81B-P13, ICMB 01222-P1, ICMB 95333-P1, ICMB 95333-P5 and IPC 804-P4 were found to be highly susceptible (>80 % DMI) in screening against three Indigenous pathogen isolates from Gujarat (Sg445), Haryana (Sg519) and Rajasthan (Sg526), while 863B-P2, AIMP 92901-S1-183-2-2-B-P08 and AIMP 92901-S1-15-1-2-B-P03 were resistant (<10% DMI) to test isolates. Some parents exhibited different levels of DM incidence to pathogen - isolates.

Key words: *Inoculation; Isolates; Pathogenicity; Screening; Resistance;*

Abbreviations: *DMI_ Downy mildew incidence; RH_ Relative humidity; Sg_ Sclerospora graminicola.*

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[9]. Pearl millet, with 8-19% seed protein content and 56-65% carbohydrates is nutritionally superior to rice, sorghum and maize.

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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%[17]. 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[23]. During the 1970s- 80s several downy mildew epidemics occurred in India resulting in considerable yield losses and withdrawal of several hybrids from cultivation[12,16]. Currently, over 70 different hybrids are being grown in India[23] 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[19,10,23]. Existence of mating types and their frequency greatly contribute towards the development of new recombinants in the pathogen populations[7] . Evolution of host-specific virulence in pearl millet downy mildew is well documented[24,11,8]. 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 forty germplasm lines which are regularly used in development of mapping populations at ICRISAT and five control entries *viz.,* 7042(S), IP18292, 843B, 843-22B and ICMP 451. 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[22]. 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[18].

The Experimental material of 40 parental lines and 5 checks 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

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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

Among the parental lines under study, ICMP 85410-P7, LGD-1-B-10, Tift 23DB-P1-P5, H77/833-2-P5, H77/833-2, Tift 238D1, ICMB 89111-P6, 81B-P13, ICMB 01222-P1, ICMB 95333-P1, ICMB 95333-P5 and IPC 804-P4 were found to be highly susceptible (>80 % DMI) in DM screening against three pathogen isolates from Gujarat (Sg445), Haryana (Sg519) and Rajasthan (Sg526), while 863B-P2, AIMP 92901-S1-183-2-2-B-P08 and AIMP 92901-S1-15-1-2-B-P03 were resistant (<10% DMI) to test isolates (Table1).

Very high downy mildew incidence levels (80-100%) were observed in WSIL-P8, ICMB 90111-P2, Jakrana S8-28-2-P4, RIB 335/74-P1 and 81 B-P8 against Sg445 and Sg519 but susceptible (50-80%) to the isolate Sg526. The parents 81B-P6 and ICMB 01222-P5 exhibited high susceptibility towards the pathotypes Sg445 and Sg519 but recorded moderate susceptibility (25-50%) towards Sg526. P310-17-Bulk and IP 18293 had moderate DM incidence from Gujarat and Haryana isolates, while resistance to the isolate from Rajasthan. P14499-2-P1 and ICMB 89111-P5 were found to be highly susceptible against Sg445 and Sg519 but exhibited resistance towards the isolate Sg526. The lines ICMB 89111-P2 and ICMB 95444 have shown high susceptibility to Sg445, Sg519 and moderate levels of resistance (10-25%) to Sg526 (Table.1). The parent PRLT2/89-33 has shown susceptibility with Sg445 and Sg519 in contrary it has shown resistance to Sg526. W504 -1-1 was resistant to Sg445, but moderate resistance to Sg519 and Sg526. PT 732B-P2 has shown resistance to Sg445 and Sg519, while it is moderately susceptible to Sg526 and the parent ICMB 96222 was moderately resistant to Sg445 where as high DM incidence was exhibited with Sg519 and Sg526. ICMP 451-P6 was recorded high susceptibility with Sg445, but it has shown susceptibility with Sg519 and Sg526.

Some parents exhibited different levels of DM incidence with three isolates of the pathogen *Sclerospora graminicola* under

investigation. ICMP 451-P8 has shown high susceptibility, susceptibility and moderate resistance to Sg445, Sg519 and Sg526, respectively. The parent 841 B-P3 exhibited moderate susceptibility to Sg445, moderate resistance to Sg519 and resistance to Sg526. Jakhrana S35-2-P2 and RIB 334/74-P1 had shown susceptibility, high susceptibility and moderate susceptibility to Sg445, Sg519 and Sg526 respectively. Moderate resistance was observed with Sg445 in ICMB 90111-P6, but to Sg519 and Sg526 shown moderate susceptibility and resistance, respectively. ICMB 90111-P5 exhibited DM resistance with Sg445, but with Sg519 shown susceptibility, with Sg526 had moderate resistance. High DM incidence was observed with Sg526, moderate susceptibility with Sg445 and susceptibility with Sg519 in ICMS 8511-S1-17-2-1-1-B-P03. Similar type of phenotyping studies were conducted[27,5,20,1]. Although different genotypes shown differential reaction to the Downy mildew incidence level, the pathotypes Sg445 (69.8%) and Sg519 (70.45%) are showing high virulence compare to Sg526 (49.28%) fig-1. The Downy mildew incidence levels of the pathotypes Sg445 and Sg519 are on par and they are significantly different from the pathotype Sg526 (Table 2).

Table 1: The mean downy mildew incidence percentage (DMI%) of 40 mapping population parental lines along with 5 checks against three virulent isolates of pathogen, *Sclerospora graminicola* under greenhouse conditions at Patancheru, India, 2013

Ent. No	Parents and Controls	DMI%			Comments
		Sg445-1	Sg519-1	Sg526-1	
1	ICMP 85410-P7	85.75	91.95	80.22	HS
2	LGD-1-B-10	97.07	100.00	100.00	HS
3	Tift 23DB-P1-P5	100.00	100.00	100.00	HS
4	WSIL-P8	88.97	96.47	78.53	HS to S
5	81B-P6	99.07	99.40	35.37	HS to MS
6	ICMP 451-P8	85.98	57.38	13.00	HS to MR
7	ICMP 451-P6	84.95	51.32	51.92	HS to S
8	H77/833-2-P5	91.63	92.27	89.70	HS
9	PRLT2/89-33	55.15	76.60	0.00	S to R
10	H77/833-2	94.38	92.48	91.28	HS
11	W504-1-1	7.58	11.62	10.05	MR to R
12	P310-17-Bulk	38.32	39.37	2.90	MS to R
13	Tift 238D1	100.00	99.28	100.00	HS
14	IP 18293	23.60	12.92	1.63	MS to HR
15	PT 732B-P2	8.87	1.28	46.15	MS to HR
16	P1449-2-P1	82.27	82.75	1.38	HS to HR
17	841B-P3	32.82	23.93	1.05	MS to HR
18	863B-P2	8.63	0.83	1.33	R to HR
19	ICMB 89111-P2	99.55	97.12	11.85	HS to MR
20	ICMB 90111-P2	87.15	95.02	77.68	HS to S
21	ICMB 89111-P5	98.88	99.43	7.30	HS to R
22	ICMB 90111-P5	6.63	51.93	15.02	S to R
23	ICMB 89111-P6	97.53	97.65	95.32	HS

24	ICMB 90111-P6	10.72	36.38	7.67	MS to R
25	Jakrana S8-28-2-P4	94.13	96.90	75.53	HS to S
26	RIB 335/74-P1	86.43	92.35	63.78	HS to S
27	Jakhrana S35-2-P2	79.25	80.17	43.35	HS to MS
28	RIB 334/74-P1	55.55	82.48	45.10	HS to MS
29	ICMS 8511-S1-17-2-1-1-B-P03	44.85	64.02	82.22	HS to MS
30	AIMP 92901-S1-183-2-2-B-P08	5.70	0.00	2.38	R to HR
31	81B-P13	100.00	99.17	92.65	HS
32	AIMP 92901-S1-15-1-2-B-P03	2.27	1.27	4.12	HR
33	ICMB 01222-P1	95.22	95.28	91.07	HS
34	ICMB 95333-P1	100.00	92.58	87.35	HS
35	ICMB 01222-P5	97.42	98.80	45.18	HS to MS
36	ICMB 95333-P5	98.12	94.78	91.65	HS
37	IPC 804-P4	96.95	97.55	92.32	HS
38	81B-P8	100.00	98.48	62.13	HS to S
39	ICMB 95444	97.87	89.97	24.80	HS to MR
40	ICMB 96222	11.95	5.42	2.22	MR to HR
41	7042S @ C	98.60	96.68	95.22	HS
42	843B @ C	97.75	94.25	54.10	HS to S
43	843-22B @ C	4.13	1.35	0.00	HR
44	ICMP 451 @ C	96.18	84.37	54.33	HS to S
45	IP 18292 @ C	94.28	97.07	88.83	HS

Highly susceptible - DMI>80%, Susceptible 50%-80% DMI, Moderately susceptible 25% -50% DMI, Moderately resistant 10% - 25% DMI and Resistant < 10% DMI

Table2: ANOVA of the Experiment in RBD with 3 replications of 40 parents and 5 checks

	Sg445-1	**Sg519-1**	**Sg526-1**
Mean	69.83	70.45	49.28
F-statistic for entry	161.3572**	203.4467**	195.0139**
SED	4.10	3.59	3.83
CD (critical difference)	8.19	7.19	7.66
Error mean square (EMS)	25.17	19.36	21.99
CV%	7.19	6.25	9.52
Genetic variance	1345.66	1306.52	1422.35
Error variance	25.17	19.36	21.99
Heritability	0.99	1.00	0.99

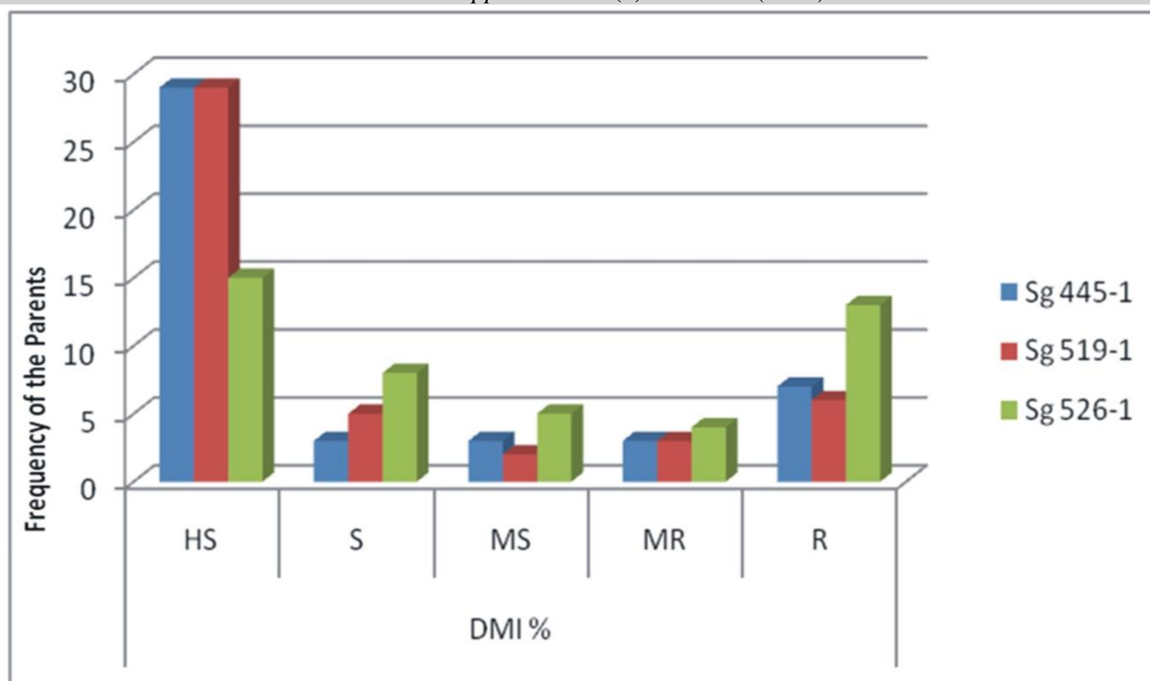

Fig. 1: Frequency distribution of downy mildew disease incidence (%) in 40 parental lines along with 5 checks screened under green house conditions at Patancheru against Sg 445-1, Sg 519-1 and Sg 526-1

DISCUSSION

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* 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[15,21].

On-farm surveys in the hybrid-intensive states of India during the past several years have indicated increased susceptibility of a hybrid when grown in the same field for more than three consecutive crop seasons suggesting emergence/selection of new virulence traits over time at the same location[19,23]. The major change in disease incidence (%) of a pearl millet line over time at the same location was considered as reflection of virulence shift in the pathogen population. This is based on the basic assumptions that variables, such as environmental factors and inoculum load, were optimal for disease development and that the seed of each pearl millet line was genuine at both times of testing.

Breeding crop varieties with durable resistance to diseases is made difficult by the variability in the pathogen populations[6]. Genetic resistance in a cultivar at one location may not function at another location because of the differences in virulence in the pathogen populations[2,4,26]. Differential host varieties are useful in the analysis of pathogen variability at various locations on the basis of clearly visible resistant and susceptible reactions[26]. According to Flor's 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[3].

In the case of *S. graminicola* – pearl millet system, the limitation has been the unavailability of well-defined differential lines. Because of the highly heterogeneous and heterozygous nature of pearl millet and highly

variable *S. graminicola* populations, it has been difficult to define genes for resistance and genes for virulence in the system. However, there are pearl millet inbred lines that serve as putative differentials to discern the virulence patterns in *S. graminicola* populations to a reasonable level[25].In the pearl millet downy mildew pathosystem, disease incidence levels indicate quantitative differences for virulence in the pathogen and resistance in the host. Quantitative variation in *S. graminicola* isolates was studied by calculating the virulence index from two independent measures of pathogenicity, disease incidence and latent period. Variation in the pathogen population for virulence on the host genotypes is required for the selection of host-specific virulence[24].

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[13,14].

Among various control entries 7042(S) showed 95.22-98.6% DMI and IP 18292 was found to possess 88.83 - 94.28% DMI across all Indian isolates of pathogen. Another susceptible control entry (843B) exhibited DMI values ranging from 94.25% to 97.75% across these Indian pathogen populations against which it was screened except that from Rajasthan (54.10 %). A very highly resistant reaction was observed for resistant control 843-22B in screens against all three Indian pathogen populations (DMI values ranges from 0% to 4.13 %). ICMP 451 exhibited 84.37 - 96.18 % DMI against Sg445 and Sg519 except with Sg526 was showed 54.33 DMI%.

The above results for different pathogen isolates of *S.graminicola* showed significant differences in the genetic structure of pathogenicity and virulence in pathogen isolates from different origins. This fact has been supported by previous studies by where the differences between pathogen isolates from India and Africa were found.

CONCLUSION

The results of this study can be used to identify the parental lines which can be useful in effective downy mildew resistance breeding in pearl millet 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. Although these accessions used in this study have exhibited good levels of resistance to individual pathotypes and these could be strategically utilized in resistance breeding to effectively manage the disease and enhance productivity of pearl millet.

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