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Variability in Virulence of Pearl millet Downy Mildew Pathogen Isolates against RIL Mapping Population of Pearl millet

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ABSTRACT

Downy mildew is the most destructive disease of pearl millet causing huge grain yield losses and reducing life span of hybrids. Rapid evolution of new isolates due to genetic recombination by sexual stages in its life cycle makes it important to identify and map new sources of resistance for its use in pearl millet breeding program. In the present investigation, a total of 187 F_8 RILs of the cross ICMB 89111-P6 × ICMB 90111-P6 were used for screening against virulent isolates of the downy mildew pathogen, Sclerospora graminicola from Gujarat (Sg445), Haryana (Sg519) and Rajasthan (Sg526) states of India. Downy mildew disease screening of RIL population was done at Controlled Environment Research Facility, International Crops Research Institute for the Semi-Arid Tropics, Patancheru where disease incidence was ranged from 0 to 100% with high heritability. The individual comparisons of average downy mildew disease incidence of 187 F_8 RILs made among three pathogen isolates revealed that the pathogen isolate of S. graminicola from Banaskantha (Sg445) caused the greatest disease incidence (59.54%) followed by the pathogen isolate from Rewadi (Sg519) with 40.55% (Fig.1). The pathogen isolate from Jodhpur (Sg526) with mean DMI 37.47% was observed to be the least virulent pathogen isolate used in this study.

***Key words**: Downy mildew, RIL mapping population, Heritability and Virulence.*

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INTRODUCTION

Pearl millet (*Pennisetum glaucum* [L.] R. Br.), also known as spiked millet, bajra is the fifth most important cereal crop in the world. It is a highly tillering, cross-pollinated, tropically-adapted C_4 cereal. It is the sixth most important cereal in the world. In India it is cultivated in Rajasthan, Uttar Pradesh, Gujarat, Haryana, Maharashtra, Karnataka and Andhra Pradesh with a total area of 7.20 million hectares with production of 8.74 million tonnes and the national average productivity is 1214 kg ha^{-1} (Directorate of Economics and Statistics, 2012-13). Among diseases affecting pearl millet, downy mildew, also known as the green ear disease, is most devastating. 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). It is caused by systemic infection by the obligate biotrophic, oomycete *Sclerospora graminicola* [(Sacc). Schroet]. *S. graminicola* is known to be a highly variable pathogen

because the existence of sexual stages in its lifecycle which helps the pathogen undergo rapid genetic recombination leading to the emergence of new pathotypes and races with high degree of spatial and temporal variation for virulence (Thakur *et al.* 2009). Evolution of more virulent pathotypes of *S. graminicola* in the recent past has resulted in the susceptibility of pearl millet accessions hitherto resistant to earlier pathotypes. Therefore, monitoring stability of resistance in the breeding lines against more virulent populations is an important component of resistance breeding in pearl millet for the success of improved varieties and hybrids in the farmer fields.

As the host is a crop of poor and marginal areas, the use of resistant cultivars is the most appropriate, efficient, environmental friendly and economical means for the control of pearl millet downy mildew. Therefore, breeding for improvement of yield and resistance to downy mildew has been a prime concern of pearl millet breeders. Although in the past elite cultivars resistant to downy mildew have been developed worldwide through conventional breeding, rapid evolution of new virulent isolates of downy mildew pose serious challenges to the breeding community in phenotypic selection. With this background, the current study was undertaken to screen the mapping population against virulent isolates of downy mildew pathogen which is invariably useful in identification of QTLs and candidate genes governing resistance to the DM.

MATERIALS AND METHODS

Plant Material: A total of 187 RILs along with parents (ICMB 89111-P6, ICMB 90111-P6) were obtained from pearl millet breeding unit, Dry land cereals, ICRISAT, Patancheru.

Downy Mildew Pathogen Isolates: Three new isolates i.e. Sg445 from Banaskantha, Gujarat; Sg519 from Rewadi, Haryana and Sg526 from Jodhpur, Rajasthan collected during 2009 and 2010 from the A_1 zone in India by Sharma *et al*. 2014 were used in the present study.

Disease Phenotyping: An effective greenhouse screening technique developed at ICRISAT (Singh *et al*. 1993) was employed in the present study for identifying resistance in mapping population parents and mapping population to different pathotypes of *S. graminicola* in a relatively small space and shorter time period under uniform conditions of disease development. This screening technique minimizes escape as every seedling is uniformly inoculated, can be operated throughout the year and it is rapid, reliable and cost-effective technique compared to field screening. Phenotyping of mapping population of 187 RIL entries along with their parental lines and control entries such as 7042(S) and IP 18292 was done during *Kharif,* 2013. This mapping population was sown in a randomized block design with three replications under greenhouse conditions at ICRISAT, Patancheru. Thirty five seeds from each genotype were sown in 12 cm diameter plastic pots, filled with a potting mixture and these pots are maintained in the greenhouse at 35°C till seedling emergence.

Artificial disease epiphytotics were created by spraying the inoculum having sporangial concentration of 1×10^6 ml^{-1} on the seedlings at the coleoptile to first-leaf stage using pneumatic atomizer till run-off ensuring that every seedling has received uniform inoculum load, then the 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. Disease scoring was done at 14 days after shifting to mist chamber. The disease incidence was scored as: highly susceptible - DMI >80%, susceptible - 50 to 80% DMI, moderately susceptible - 25 to 50% DMI, moderately resistant - 10 to 25% DMI and resistant <10% DMI.

RESULTS

Downy mildew phenotyping of mapping population

Phenotyping of mapping population was done during *Rainy Season*, 2013 under greenhouse conditions at ICRISAT. Male parental line ICMB 90111-P6 was highly resistant and exhibited very low downy mildew incidence (DMI) against two pathogen isolates (Sg519: 1.67% and Sg526: 7.66%)) and moderately resistant to pathogen isolate (Sg445: 10.73%). Female parental line ICMB 89111-P6 was highly susceptible to all the three pathogen isolates (Sg445: 97.54%, Sg519: 97.65% and Sg526: 95.33%).

Analysis of variance for screens against these three individual isolates from India is summarized in Table 1. The individual comparisons of average downy mildew disease incidence of 188 F_8 RILs made among three Indian isolates revealed that the isolate of S *graminicola* from Banaskantha (Sg445) caused the greatest disease incidence (59.54%) followed by the isolate from Rewadi (Sg519) with 40.55%. The isolate from Jodhpur (Sg526) with mean DMI 37.47% was observed to be the least virulent isolate used in this study. Analysis of variance of these phenotyping data revealed a general trend of high heritability (Broad Sense) estimates for DMI for screens against the Rewadi (0.97) and Jodhpur (0.95) isolates. The lowest broad sense heritability value for DMI was recorded from screening against Banaskantha isolate

(0.91). In the same way high broad sense heritability estimates were recorded for total plants (0.6) and number of diseased plants (0.9) for the isolates from Rewadi and Jodhpur (TP: 0.6 and DP: 0.88). The lowest broad sense heritability estimate was recorded for total plants (0.59) and number of diseased plants (0.81) for the isolate from Banaskantha.

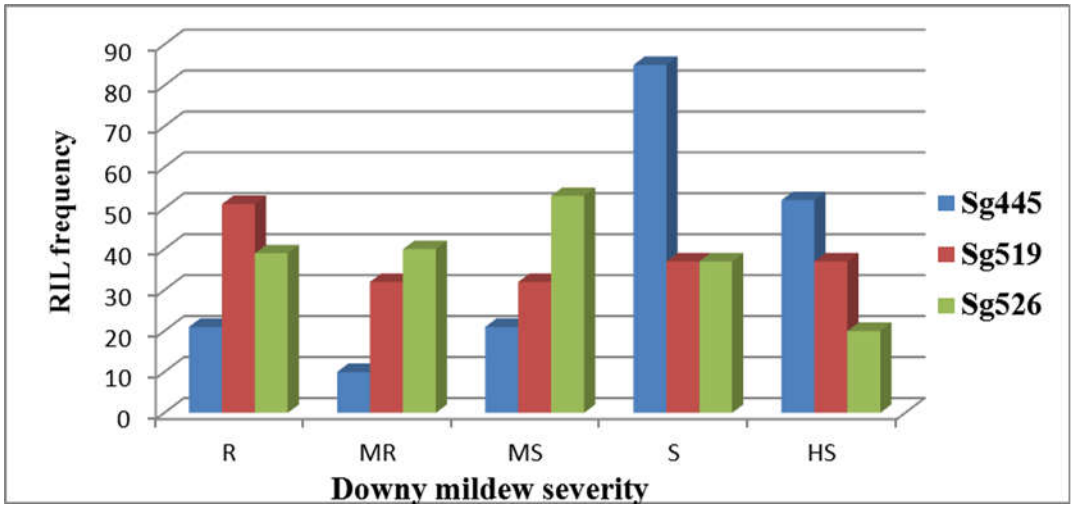
Table-1. Individual summaries of DM screens of 188 F_8 RIL mapping population progenies against three virulent isolates of *Sclerospora graminicola*

Summary	Banaskantha (Sg445)			Rewadi (Sg519)			Jodhpur (Sg526)		
	DMI%	TP	DP	DMI%	TP	DP	DMI%	TP	DP
Mean	59.54	23.86	16.41	40.55	23.12	9.94	37.47	23.65	8.86
SEm	4.89	3.13	2.67	2.73	3.06	1.83	2.89	3.13	1.77
SEd	6.92	4.43	3.77	3.86	4.33	2.6	4.08	4.42	2.5
CV (%)	14.54	22.95	28.4	11.64	22.94	32.03	13.3	22.93	34.64
CD (0.05)	13.61	8.72	7.42	7.59	8.51	5.11	8.03	8.7	4.93
h^2 (BS)	0.91	0.59	0.81	0.97	0.6	0.9	0.95	0.6	0.88

Table-2. Performance of pearl millet RILs derived from cross (ICMB 89111-P6 x ICMB 90111-P6) for pearl millet downy mildew resistance under glasshouse conditions with artificial inoculation

Percentage severity	Reaction	Number of RILs		
		Sg445	Sg519	Sg526
<10	Resistant	21	51	39
10-25	Moderately resistant	10	32	40
25-50	Moderately susceptible	21	32	53
50-80	Susceptible	85	37	37
80-100	Highly susceptible	52	37	20

Fig-1. Frequency distribution of downy mildew severity (%) among F_8 RIL progenies from the Pearl millet cross ICMB 89111-P6 x ICMB 90111-P6.



Where **R** - resistant, **MR** - Moderately resistant, **MS** - Moderately susceptible, **S** - Susceptible and **HS** - Highly Susceptible

DISCUSSION

The most economic and efficient strategy for the management of downy mildew of pearl millet is host plant resistance. 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 (Sivaramakrishnan *et al.,* 2003; Thakur *et al.,* 2004).

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. 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 (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). For the analysis of pathogen variability at various locations differential host varieties are useful on the basis of clearly visible resistant and susceptible reactions.

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 (Thakur, 1995). 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.

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 %).

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 Azhaguvel (2001) 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 indicates the variability in the virulence level of pathogen isolates for the same genotypes, 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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