

# Inheritance and Allelic Relationship Among Downy Mildew Resistance Genes in Pearl Millet

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## Abstract

Pearl millet downy mildew (DM), caused by *Sclerospora graminicola*, is of serious economic concern to pearl millet farmers in the major crop-growing areas of the world. To study the inheritance and allelic relationship among genes governing resistance to this disease, three DM-resistant pearl millet lines (834B, IP 18294-P1, and IP 18298-P1) and one susceptible line (81B) were selected on the basis of disease reaction under greenhouse conditions against two isolates of *S. graminicola* (Sg 526-1 and Sg 542-1). Three resistant parents were crossed with the susceptible parent to generate F<sub>1</sub>, F<sub>2</sub>, and backcross BC<sub>1</sub>P<sub>1</sub> (susceptible parent × F<sub>1</sub>) and BC<sub>1</sub>P<sub>2</sub> (resistant parent × F<sub>1</sub>) generations for inheritance study. To carry out a test for allelism, the three resistant parents were crossed with each other to generate F<sub>1</sub>

and F<sub>2</sub> generations. The different generations of these crosses were screened for disease reaction against two isolates (Sg 526-1 and Sg 542-1) by artificial inoculation under greenhouse conditions. The segregation pattern of resistance in the F<sub>2</sub> and corresponding backcross generations revealed that resistance to DM is controlled by a single dominant gene in 834B and IP 18294-P1 and by two dominant genes in IP 18298-P1. A test for allelism inferred that a single dominant gene for resistance in 834B is nonallelic to that which governs resistance in IP 18294-1, whereas one of the two dominant genes for DM resistance in IP 18298-P1 against the test isolates is allelic to the gene for DM resistance in 834B and a second gene is allelic to the resistance gene present in IP 18294-P1.

Pearl millet downy mildew (DM) or green ear disease, caused by the oomycete *Sclerospora graminicola* (Sacc.) J. Schröt., was first reported by Butler (1907) in India and described as a disease of ill-drained lands. It is a highly destructive and widespread disease in most of the pearl-millet-growing areas of Asia and Africa (Sharma et al. 2014). The disease was considered to be a minor disease in India till 1970 due to fewer incidences on local cultivars; however, it reached epidemic levels during the mid-1970s to 1980s, when only a few single-cross hybrids were cultivated on a large scale. The first epidemic of DM occurred in 1971 on a popular pearl millet hybrid, HB 3, and resulted in severe grain loss of about 4.6 million metric tons (Singh et al. 1993). The estimated annual grain yield loss due to DM is approximately 20 to 40% but could be much higher, up to 80%, under favorable conditions of high relative humidity, moderate temperature, and widespread use of the same cultivar across fields (Singh and Singh 1987; Thakur et al. 1999). Among control measures, the most important one is the use of resistant (R) cultivars, due to its high efficiency, low cost, and no harmful impact on environment (Thakur et al. 2004).

The continuous emergence of host-specific virulences in *S. graminicola*, due to a high degree of pathogenic variability, leads to frequent breakdown of disease resistance and poses a continuous challenge to pearl millet resistance breeding (Pushpavathi et al. 2006; Sastry et al. 2001; Thakur et al. 1992; Werder and Ball 1992). Therefore, pyramiding of resistance genes in a single genotype or identification of a single genotype with different resistance genes conferring resistance to multiple pathotypes would be the effective management strategy to increase the durability of resistance. Attempts have been made to identify several sources of resistance against one or multiple pathotypes and, based on resistance sources, several R hybrids and varieties have been released for general cultivation in India (Hash et al. 2006; Sharma et al. 2015). In addition, the inheritance of DM resistance in pearl millet has been interpreted by various workers in terms of simple and complex interactions. The DM resistance has

generally been reported to be governed by a dominant gene (Appadurai et al. 1975; Singh and Talukdar 1998); a recessive gene (Pethani et al. 1980; Singh et al. 1980); one, two, or more genes (Appadurai et al. 1975; Gill et al. 1978; Joshi and Ugale 2002); and multiple genes with epistatic effect (Deswal and Govila 1994). However, limited information on the allelic relationship among resistance genes is available. The inconsistency of gene effects over crosses could be clarified with the analysis of allelic diversity of resistance genes in pearl millet to DM and it would be more helpful in understanding that interaction between host and pathogen that will further help in the development of management strategies. Thus, in the present study, an attempt has been made to decipher the mode of inheritance of DM resistance genes and the allelic relationship between them.

## Materials and Methods

**Pearl millet genotypes and pathogen isolates.** Seed of pearl millet genotypes were taken from genetic stocks being maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Selected pearl millet lines were further selfed for three consecutive generations to obtain true inbreds. *S. graminicola* isolates being maintained at the Cereals Pathology Lab, ICRISAT were used to screen the pearl millet lines. The use of single-spore culture is highly recommended for inheritance and allelism studies to counter variability within pathogen populations. Hence, single-zoospore isolates Sg 526-1 (derived from pathotype Sg 526, collected from Jodhpur, Rajasthan) and Sg 542-1 (derived from pathotype Sg 542, collected from Aurangabad, Maharashtra) were selected for greenhouse screening. These single-zoospore isolates were used to screen pearl millet lines to select R and susceptible (S) lines for crossing. Disease incidence (DI) was recorded 14 days after inoculation as percent infected plants. The lines with ≥90% DI and ≤10% DI were selected as S and R parents, respectively. Based on DI against Sg 526-1 and 542-1, 834 B, IP18294-P1 (a single plant selection from IP 18294), and IP18298-P1 (a single plant selection from IP 18298) were selected as R and 81B as S parents to generate crosses for inheritance and allelism studies. To understand whether the same or different genes for resistance are operative in the R genotypes used in this study, test for allelism was conducted by crossing all of the R parents (834B, IP 18294-P1, and IP 18298-P1) with each other. Description of the pearl millet genotypes used in this study is presented in Table 1.

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Accepted for publication 22 December 2017.

**Generation of progenies segregating for DM resistance.** Staggered sowings were carried out to synchronize flowering between S and R parents. Seedlings of both R and S parents were inoculated with the *S. graminicola* isolate and disease-free seedlings of the R parents were transplanted into a 25-cm-diameter pot (4 plants/pot) and used in crossing. The S plants of 81B were recovered by spraying Ridomyl MZ 72 WP (2 g liter<sup>-1</sup>) and used as S parents. For inheritance studies, three S × R F<sub>1</sub>s (81B × 834B, 81B × IP 18294-P1, and 81B × IP 18298-P1) were generated. To carry out allelism test, three R × R F<sub>1</sub>s (834B × IP 18294-P1, 834B × IP 18298-P1, and IP 18294-P1 × IP 18298-P1) were produced in half diallel fashion without reciprocal crosses during March to June 2014. All of the crosses were made in a greenhouse. In the subsequent postrainy season during November 2014 to February 2015, each F<sub>1</sub> (8 to 10 panicles) was selfed using parchment paper bags for the production of F<sub>2</sub> seed. Single-head pollen of each F<sub>1</sub> plant was used to pollinate the respective S and R parents to develop backcross populations BC<sub>1</sub>P<sub>1</sub> (S parent × F<sub>1</sub>) and BC<sub>1</sub>P<sub>2</sub> (R parent × F<sub>1</sub>).

**Inoculum preparation.** The systemically infected leaves of S line 7042S inoculated with Sg 526-1 and Sg 542-1 and grown in isolation chambers were collected, excised into pieces, washed under running tap water to remove old sporangia, wiped dry with tissue paper, placed by their abaxial surfaces up in humidity chambers lined with moist blotting paper, and incubated in darkness at 20°C for 6 h to induce sporulation. Sporangia of each isolate were harvested separately in ice-cold (4°C) distilled sterilized water from the sporulating leaves using a soft camel-hair brush and filtered through a double-layered muslin cloth. The concentration of sporangia was measured using a hemocytometer and adjusted to 1 × 10<sup>6</sup> ml<sup>-1</sup> before inoculation.

**Sowing, inoculation, and disease evaluation.** For the inheritance study, seed of parents and F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>1</sub>, and BC<sub>1</sub>P<sub>2</sub> generations of each cross were sown in 15-cm-diameter pots (30 to 35 seeds/pot) filled with a sterilized soil-sand-farmyard manure mix (2:1:1) and placed in a greenhouse bay maintained at 30 ± 2°C. For the allelism study, seed of parents and F<sub>1</sub> and F<sub>2</sub> generations of each R × R cross were sown in plastic pots as described above for the inheritance study. The 48-h-old seedlings of parents and six F<sub>1</sub> (three S × R and three R × R), three BC<sub>1</sub>P<sub>1</sub>, three BC<sub>1</sub>P<sub>2</sub>, and six F<sub>2</sub> (three S × R and three R × R) populations were spray inoculated with an aqueous sporangial suspension (approximately 1 × 10<sup>6</sup> ml<sup>-1</sup>) of two isolates (Sg 526-1 and Sg 542-1) of *S. graminicola* separately using an atomizer and covered immediately with moist polyethylene sheet to provide >90% relative humidity. Inoculated seedlings were incubated in the dark at 20°C for 24 h, then transferred to greenhouse benches maintained at 25 ± 2°C under mist to facilitate disease development (Thakur et al. 2011). Observations on individual seedlings were recorded at 14 days after inoculation; the plants showing DM symptoms were classified as S and healthy plants were categorized as R.

**Statistical analysis.** The observed ratios of R to S plants in the segregating generations (F<sub>2</sub> and BC<sub>1</sub>) in the greenhouse were compared with theoretical ratios using a  $\chi^2$  test. The  $\chi^2$  test ( $P \leq 0.05$ ) was used to test the segregation ratio of the phenotypic classes by using the program GENES (Cruz 2001).

## Results

**Inheritance of DM resistance.** The results of inheritance studies for each cross against two isolates, Sg 526-1 and Sg 542-1, are summarized in Tables 2 and 3, respectively. The S parent 81B was severely infected, showing approximately 90% DI, whereas plants of R parents

834 B and IP 18298-P1 were mostly disease free and IP 18294-P1 exhibited <10% DM incidence against both isolates. In the cross 81B × 834B, 108 F<sub>1</sub> plants, 850 F<sub>2</sub> plants, 383 BC<sub>1</sub>P<sub>1</sub> (backcross with S parent) plants, and 416 BC<sub>1</sub>P<sub>2</sub> (backcross with R parent) plants were screened against Sg 526-1; all of the F<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> plants exhibited resistance to Sg 526-1, which indicated that resistance in 834B to Sg 526-1 is governed by dominant gene(s). In the F<sub>2</sub> generation, 651 plants were R and 199 were S, which fitted the segregation ratio of 3:1 R/S. Of 383 BC<sub>1</sub>P<sub>1</sub> plants, 196 were R whereas 187 exhibited a susceptible reaction, which indicated a 1:1 R/S segregation ratio. Similar results were obtained for different generations of this cross when screened against Sg 542-1; all F<sub>1</sub> plants (106) and most of BC<sub>1</sub>P<sub>2</sub> plants exhibited a resistant reaction whereas segregations were observed in F<sub>2</sub> and BC<sub>1</sub>P<sub>1</sub> plants (677 R and 228 S plants in F<sub>2</sub>, falling in a 3:1 R/S ratio, and 176 R and 159 S plants in BC<sub>1</sub>P<sub>1</sub>, with a 1:1 R/S ratio) (Table 3). In the second cross, 81B × IP 18294-P1 screened against Sg 526-1 (Table 2), all 120 plants of F<sub>1</sub> generation and 357 plants of BC<sub>1</sub>P<sub>2</sub> generation exhibited a resistant reaction, indicating the dominant nature of the resistance gene in IP 18294-P1 to Sg 526-1, as observed in 834 B, whereas F<sub>2</sub> and BC<sub>1</sub>P<sub>1</sub> generations segregated into R (506 plants in F<sub>2</sub> and 218 plants in BC<sub>1</sub>P<sub>1</sub>) and S (151 plants in F<sub>2</sub> and 191 plants in BC<sub>1</sub>P<sub>1</sub>), with the best-fit ratio of 3:1 R/S in F<sub>2</sub> generation and 1:1 R/S in BC<sub>1</sub>P<sub>1</sub> generation (Table 2). Similar results were observed for this cross when screened against Sg 542-1 for different generations. The segregation ratios of 3:1 R/S in F<sub>2</sub> (558 R and 172 S) and 1:1 R/S in BC<sub>1</sub>P<sub>1</sub> (175 plants R and 148 S) generations were obtained (Table 3). The third cross, 81B × IP 18298-P1, showed different segregation patterns in F<sub>2</sub> and BC<sub>1</sub>P<sub>1</sub> than the other two crosses. A segregation ratio of 15:1 R/S (735 R and 52 S plants) in the F<sub>2</sub> generation and 3:1 R/S (311 R and 111 S plants) in the BC<sub>1</sub>P<sub>1</sub> generation was observed against Sg 526-1 (Table 2). Similar results were found when different generations of this cross were screened against Sg 542-1; all F<sub>1</sub> (122) and BC<sub>1</sub>P<sub>2</sub> plants (287) exhibited a resistant reaction whereas segregations were observed in F<sub>2</sub> and BC<sub>1</sub>P<sub>1</sub> generations (823 R and 36 S plants in F<sub>2</sub> and 281 R and 88 S plants in BC<sub>1</sub>P<sub>1</sub>), with the best fit ratio of 15:1 R/S in F<sub>2</sub> generation and 3:1 R/S in BC<sub>1</sub>P<sub>1</sub> generation (Table 3). The results of the DM screen indicated that resistance to Sg 526-1 and Sg 542-1 in 834 B and IP 18294-P1 is governed by a single dominant gene, whereas two dominant genes impart resistance in IP 18298-P1 against these isolates.

**Test for allelism.** The results of DM screening of the F<sub>2</sub> generation of 834B × IP 18294-P1 (R × R) against Sg 526-1 are presented in Table 4. In total, 690 F<sub>2</sub> plants derived from 834B × IP 18294-P1 were screened that segregated into 640 R and 50 S plants. This indicated that genes governing resistance in these two genotypes to *S. graminicola* isolate Sg 526-1 are different. The F<sub>2</sub> plants derived from this cross again segregated for resistance (356 R and 27 S plants) when screened against another isolate, Sg 542-1 (Table 5). In contrast, no segregation was observed in F<sub>2</sub> plants derived from 834B × IP 18298-P1 and IP 18294-P1 × IP 18298-P1 crosses against both Sg 526-1 and Sg 542-1 (Tables 4 and 5), indicating an allelic relationship between genes for DM resistance in IP 18298-1 with those present in 834B and IP 18294-1. In all of the crosses, F<sub>1</sub> plants were resistant to both isolates.

## Discussion

Pearl millet is a diploid (2n = 2x = 14) cereal crop and parent materials selected for this study were mostly homozygous inbred lines

**Table 1.** Description of the pearl millet genotypes used in the study<sup>a</sup>

Genotype	Origin	Pedigree	Special characteristics
834 B	Uganda	An S4 progeny selected from Serere 10LB	Long bristles on spikelet, purple glumes, bold seed, loose spikelet, matures early, medium height, round and dark gray seed
IP18294	Mali	...	Zebra stripes, semicompact to compact spikelet, medium maturity, tall, elliptical and brown seed
IP18298	Burkina Faso	...	Glossy leaves, semicompact to compact spikelet, medium maturity, medium height, globular and gray seed
81B	ICRISAT, India	Induced DM-resistant selection from Tift 23DB	Bushy at the seedling stage, compact spikelet, matures late, dwarf, obovate-globular and light gray seed

<sup>a</sup> ICRISAT = International Crops Research Institute for the Semi-Arid Tropics and DM = downy mildew.

developed after selfing for a number of generations. Maternal effect for DM resistance or susceptibility in pearl millet have been reported to be absent in earlier studies (Anand-Kumar et al. 1983; Yadav 1994, 1996; Yadav et al. 1993); thus, no reciprocal crosses were made in the present study. Resistance or susceptibility in the pearl millet-DM pathosystem is measured in absolute relative terms of percentage. Complete susceptibility or resistance in pearl millet is a rare phenomenon because both host and pathogen are highly out-crossable in nature. The presence of

residual variability to the extent of 1 to 5% for resistance to DM in apparently S genotypes and for susceptibility up to 15% in apparently R genotypes has been documented (Singh and Talukdar 1998). To facilitate the selection of single-plant derivatives with complete resistance and complete susceptibility, the process of selfing and single-plant selection was followed under greenhouse conditions to reduce such hidden variability. The outcrossing nature of DM pathogen *S. graminicola*, like its host pearl millet, creates complexity in the inheritance and

**Table 2.** Segregation analysis for downy mildew reaction in the populations derived from susceptible × resistant (S × R) parent crosses to *Sclerospora graminicola* isolate Sg 526-1

Cross, generations	Number of plants observed		Expected ratio <sup>a</sup>	Number of plants expected		$\chi^2$	<i>P</i> <sup>b</sup>	RF <sup>c</sup>
	R	S		R	S			
81B × 834B								
81B	20	202	0:1	...	...	...	...	
834B	204	2	1:0	...	...	...	...	1
F <sub>1</sub>	108	0	1:0	...	...	...	...	
F <sub>2</sub>	651	199	3:1	637.5	212.5	1.14	0.28	
BC <sub>1</sub> P <sub>1</sub>	196	187	1:1	191.5	191.5	0.21	0.64	
BC <sub>1</sub> P <sub>2</sub>	416	0	1:0	...	...	...	...	
81B × IP 18294-P1								
81B	20	202	0:1	...	...	...	...	
IP 18294-P1	272	24	1:0	...	...	...	...	1
F <sub>1</sub>	120	0	1:0	...	...	...	...	
F <sub>2</sub>	506	151	3:1	492.75	164.25	1.42	0.23	
BC <sub>1</sub> P <sub>1</sub>	218	191	1:1	204.5	204.5	1.78	0.18	
BC <sub>1</sub> P <sub>2</sub>	357	0	1:0	...	...	...	...	
81B × IP 18298-P1								
81B	20	202	0:1	...	...	...	...	
IP 18298-P1	161	0	1:0	...	...	...	...	2
F <sub>1</sub>	140	0	1:0	...	...	...	...	
F <sub>2</sub>	735	52	15:1	737.81	49.19	0.17	0.67	
BC <sub>1</sub> P <sub>1</sub>	311	111	3:1	316.5	105.5	0.38	0.53	
BC <sub>1</sub> P <sub>2</sub>	371	0	1:0	...	...	...	...	

<sup>a</sup> All possible phenotypic ratio were analyzed and the best fit are shown in the table.

<sup>b</sup> *P* = probability;  $\chi^2$  values are not significant at *P* ≤ 0.05.

<sup>c</sup> Resistance factors: number of dominant genes.

**Table 3.** Segregation analysis for downy mildew reaction in the populations derived from susceptible × resistant (S × R) parent crosses to *Sclerospora graminicola* isolate Sg 542-1

Cross, generations	Number of plants observed		Expected ratio <sup>a</sup>	Number of plants expected		$\chi^2$	<i>P</i> <sup>b</sup>	RF <sup>c</sup>
	R	S		R	S			
81B × 834B								
81B	23	205	0:1	...	...	...	...	
834B	201	1	1:0	...	...	...	...	1
F <sub>1</sub>	106	0	1:0	...	...	...	...	
F <sub>2</sub>	677	228	3:1	678.75	226.25	0.018	0.89	
BC <sub>1</sub> P <sub>1</sub>	176	159	1:1	167.5	167.5	0.86	0.35	
BC <sub>1</sub> P <sub>2</sub>	366	19	1:0	...	...	...	...	
81B × IP 18294-P1								
81B	23	205	0:1	...	...	...	...	
IP 18294-P1	193	7	1:0	...	...	...	...	1
F <sub>1</sub>	107	0	1:0	...	...	...	...	
F <sub>2</sub>	558	172	3:1	547.5	182.5	0.80	0.37	
BC <sub>1</sub> P <sub>1</sub>	175	148	1:1	161.5	161.5	2.257	0.13	
BC <sub>1</sub> P <sub>2</sub>	296	10	1:0	...	...	...	...	
81B × IP 18298-P1								
81B	23	205	0:1	...	...	...	...	
IP 18298-P1	198	0	1:0	...	...	...	...	2
F <sub>1</sub>	122	0	1:0	...	...	...	...	
F <sub>2</sub>	823	36	15:1	805.31	53.69	0.48	0.49	
BC <sub>1</sub> P <sub>1</sub>	281	88	3:1	276.75	92.25	0.26	0.61	
BC <sub>1</sub> P <sub>2</sub>	287	0	1:0	...	...	...	...	

<sup>a</sup> All possible phenotypic ratio were analyzed and the best fit are shown in the table.

<sup>b</sup> *P* = probability;  $\chi^2$  values are not significant at *P* ≤ 0.05.

<sup>c</sup> Resistance factors: number of dominant genes.

allelism study. The presence of heterothallism and different sexual compatibility types in *S. graminicola* further enhances the complication by generating new variability in the pathogen (Idris and Ball 1984; Michelmore et al. 1982). Sg 526 was collected from Jodhpur, Rajasthan, India in 2009 and Sg 542 was collected from Aurangabad, Maharashtra, India during 2010. Both isolates were found virulent on all nine pearl millet lines (P 7-4, P 310-17, 700651, 7042R, 852B, IP 18292, IP 18293, ICMP 451, and 7042S) of the host differential set and were selected as highly virulent pathotypes for greenhouse screening of pearl millet breeding lines bred for these ecologies.

In the present study, the F<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> plants (backcross generation with the R parents) of the S × R crosses 81B × 834B, 81B × IP 18294-P1, and 81B × IP 18298-P1 were resistant to Sg 526-1. The complete resistance in F<sub>1</sub> and BC<sub>1</sub>P<sub>2</sub> generations indicated that the resistance in these lines is governed by dominant genes. The dominant nature of resistance in pearl millet to DM has been reported earlier (Dass et al. 1984; Singh and Talukdar 1998). The R and S plants in F<sub>2</sub> generations of the crosses 81B × 834B and 81B × IP 18294-P1 showed a good fit for the segregation ratio of 3:1 R/S to both isolates, suggesting dominant monogenic control of DM resistance in 834B and IP 18294-P1. The corresponding BC<sub>1</sub>P<sub>1</sub> generations had good fit for a 1:1 R/S ratio, thus confirming the monogenic control of DM resistance in 834B and IP 18294-P1 against both isolates. The F<sub>2</sub> generation of the cross 81B × IP 18298-P1 exhibited a good fit for the segregation ratio of 15:1 R/S to both isolates, indicating the involvement of two dominant genes for resistance in IP 18298-P1. The corresponding BC<sub>1</sub>P<sub>1</sub> (back cross with S parent) showed the 3:1 R/S ratio expected for the digenic inheritance. DM resistance has been reported to be governed by one or two genes in different inbreds of pearl millet (Appadurai et al. 1975; Singh and Talukdar 1998). The results of this study suggest monogenic resistance in 834B and IP 18494-P1 and digenic resistance in IP 18298-P1. Nevertheless, the pattern of inheritance of genes for resistance is reported to vary against different pathotypes prevalent at different locations (Deswal and Govila 1994). The inheritance of DM resistance in a single cultivar (PPMI 519) was expressed in different ratios at two locations; complementary at Delhi (9:7) and duplicate at Villupuram, Tamil Nadu (15:1) (Deswal and Govila 1994). This could be due to the differences in the reaction of genotypes against different

pathotypes of *S. graminicola* present at the two locations. However, the same pattern of inheritance of DM resistance in the genotypes tested in this study was observed against pathotype-isolates collected from diverse ecologies. The results of this study indicate that these resistance genes could be used in breeding programs to develop DM-resistant pearl millet cultivars for cultivation in different ecologies.

For effective disease management through host plant resistance against a highly variable pathogen such as *S. graminicola*, it is essential to understand whether resistance in different genotypes against specific pathotypes of the pathogen is governed by the same or different genes (Sharma et al. 2015). In this study, F<sub>2</sub> plants derived from 834B × IP 18294-P1 (R × R) showed a good fit to the segregation ratio of 15:1 R/S against both isolates, suggesting the involvement of two independent dominant resistance genes. The segregation for resistance in F<sub>2</sub> plants derived from 834B × IP 18294-P1 indicated that the gene governing DM resistance in 834B against Sg 526-1 and Sg 542-1 is nonallelic to the resistance gene present in IP18294-P1. In contrast, no segregation was observed in F<sub>2</sub> generations derived from 834B × IP 18298-P1 and IP 18294-P1 × IP 18298-P1 crosses when screened against both isolates. This indicated an allelic relationship between genes for resistance in IP 18298-P1 with the resistance genes present in 834B and IP 18294-P1. These results further confirmed the presence of two dominant genes imparting resistance in IP 18298-P1 to *S. graminicola* because the genes for DM resistance in 834B and IP 18294-P1 were found to be different. No segregation in the F<sub>2</sub> generation of a cross of two R genotypes (PPMI 519 and PPMI 517) of pearl millet has been reported and it was inferred that the same gene for DM resistance is present in these genotypes (Deswal and Govila 1994). However, different genes for resistance in two R genotypes of pearl millet against the same pathotype of *S. graminicola* have not been reported thus far based on an allelism test.

The allelic relationship between resistance genes to both isolates of *S. graminicola* in this study indicated that 834B and IP 18294-P1 each contain one nonallelic dominant gene. These two dominant resistance genes could be present in IP 18298-P1 because the genes in IP 18298-P1 were found to be allelic to the different genes for DM resistance in 834B and IP 18294-P1. The designation of *Rsg1* has been attributed to the resistance gene identified in IP 18292 (Singh

**Table 4.** Test of allelism for genes governing downy mildew resistance in pearl millet lines 834B, IP18294-P1, and IP18298-P1 to *Sclerospora graminicola* isolate Sg 526-1

Cross <sup>b</sup>	Generation	Number of plants observed <sup>a</sup>		Expected ratio <sup>c</sup>	Number of plants expected <sup>a</sup>		χ <sup>2</sup>	P <sup>d</sup>	Allelic relationship
		R	S		R	S			
834B × IP18294-P1	F <sub>2</sub>	640	50	15:1	646.87	43.13	1.17	0.28	Nonallelic
834B × IP18298-P1	F <sub>2</sub>	866	0	...	...	...	...	...	Allelic
IP18294-P1 × IP18298-P1	F <sub>2</sub>	687	0	...	...	...	...	...	Allelic

<sup>a</sup> R = resistant and S = susceptible.

<sup>b</sup> R × R parent crosses.

<sup>c</sup> All possible phenotypic ratio were analyzed and the best fit is shown in the table.

<sup>d</sup> P = probability; χ<sup>2</sup> value is not significant at P ≤ 0.05.

**Table 5.** Test of allelism for genes governing downy mildew resistance in pearl millet lines 834B, IP18294-P1 and IP18298-P1 to *Sclerospora graminicola* isolate Sg 542-1

Cross <sup>b</sup>	Generation	Number of plants observed <sup>a</sup>		Expected ratio <sup>c</sup>	Number of plants expected <sup>a</sup>		χ <sup>2</sup>	P <sup>d</sup>	Allelic relationship
		R	S		R	S			
834B × IP18294-P1	F <sub>2</sub>	356	27	15:1	359.06	23.94	0.42	0.52	Nonallelic
834B × IP18298-P1	F <sub>2</sub>	913	0	...	...	...	...	...	Allelic
IP18294-P1 × IP18298-P1	F <sub>2</sub>	729	0	...	...	...	...	...	Allelic

<sup>a</sup> R = resistant and S = susceptible.

<sup>b</sup> R × R parent crosses.

<sup>c</sup> All possible phenotypic ratio were analyzed and the best fit is shown in the table.

<sup>d</sup> P = probability; χ<sup>2</sup> value is not significant at P ≤ 0.05.

and Talukdar 1998). However, to confirm the relationship between resistance genes in 834B and IP 18294-P1 with that of IP 18292 (*RSg1*), there is a need to conduct a test for allelism for the gene governing resistance in IP 18292 with the DM resistance genes present in 834B and IP 18294-P1 by screening against the same pathotype. Nevertheless, there is a possibility of having more DM resistance genes in these lines that could be revealed by involving more R lines in the crossing program and screening with a greater number of diverse DM pathotypes. Because the lines used in this study (IP 18294-P1, IP 18298-P1, and 834B) originated from three different countries (Mali, Burkina Faso, and Uganda, respectively), the geographical diversity of these lines could represent diversity for the resistance genes as well (Caicedo 2008). Therefore, use of resistance sources of diverse origin, with the probable different genes for resistance, in the breeding programs can help diversify and broaden the genetic base for DM resistance in pearl millet to cope up with the evolving virulences in the pathogen.

Pearl millet breeding at ICRISAT, Patancheru, India has been focused on developing and disseminating a diverse range of high-yielding, DM-resistant, trait-based breeding lines and hybrid parents (seed parents and restorer parents) for utilization in hybrid development and commercialization by the National Agricultural Research System and private seed companies (Rai et al. 2014). The large on-farm hybrid cultivar diversity has not only led to the increased grain productivity but also stemmed the large-scale DM epidemics that were frequent events prior to 1990 (Rai et al. 2006). The results of the present study have important implications for breeding programs which aim to deploy DM resistance genes or stack different genes conferring resistance to different pathotypes of DM into elite cultivars. According to Thakur et al. (2008), pyramiding of genes is a strategy to develop varieties with durable DM resistance in pearl millet. The stacking of resistance genes with major effects delays the appearance of new races of the pathogen. The basis for this stability of resistance is the decrease in pathogen fitness when a number of virulence genes are necessary to overcome the resistance of the host (Van der Plank 1984). Therefore, a potential strategy in order to maintain disease resistance for a long period of time would be the introgression of several resistance genes in a single variety. The data obtained in the present study demonstrate that the breeder should choose a number of sources having different resistance genes for gene pyramiding, in order to put together in the best possible combination of genes in new cultivars. Therefore, these varieties expressing durable resistance would be resistant to a large number of pathotypes of the pathogen over a long period of time. However, more studies are required to identify different resistance genes (nonallelic) for their spatial and temporal deployment.

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