

## Mapping Host Plant Resistance Qtls against Three Virulent Isolates of Downy Mildew Pathogen In Pearl Millet (*Pennisetum glaucum* [L.] R. Br.)

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### ABSTRACT

Downy mildew (DM) epidemics have repeatedly caused huge grain and straw production losses on pearl millet single-cross hybrids in India. The present study was undertaken to identify the QTL positions controlling DMR on the linkage map for a pearl millet mapping population of 295 F<sub>7</sub> RIL population derived from a single F<sub>1</sub> plant from plant × plant cross 81 B-P13 (susceptible) × AIMP 92901-P03 (resistant) using three isolates of the pathogen from Gujarat, Haryana and Rajasthan. A skeleton genetic linkage map of 536.8 cM (Haldane) was constructed using MapMaker/Exp version 3.0b at LOD threshold value of 3.0 using 39 scorable polymorphic loci. A total of four major DMR QTLs were identified on linkage groups of 1, 3 and 4. The inheritance of these QTLs showed AIMP 92901-P03 providing the resistant alleles. At least one DMR QTL was detected and mapped for each of the three DM isolates. After validation, marker-assisted selection (MAS) can now be used for improving DMR of elite pearl millet hybrid parental lines using polymorphic flanking markers from donor parent AIMP 92901-P03.

**Key words:** Downy Mildew, Pathogen, Hybrid, Genetic Linkage

### INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop of the semi-arid tropical regions of India and West Africa and it is the sixth most important cereal crop globally, grown in the arid and semi-arid tropical regions of Asia and Africa that are home to hundreds of millions of the world's poorest farmers. It produces grains with high nutritive value even under hot, dry conditions, on infertile soils of low water holding capacity,

where other cereal crops fail. This makes pearl millet a highly desirable crop for farmers in such harsh environments. Pearl millet, with 8-19% seed protein content and 56-65% carbohydrates is nutritionally superior to rice, sorghum and maize.

Downy mildew (DM) is the most wide spread and destructive disease of pearl millet. It is caused by *Sclerospora graminicola* [(Sacc). Schroet], first reported on pearl millet in India by Butler.

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Following the release and widespread adoption of genetically uniform pearl millet single cross hybrids in India in the late 1960s. DM became an economically important disease and the first major epiphytotic in India occurred in the early 1970s.

Molecular markers linked to resistance genes would allow resistances to different pathogen populations to be selected for at a single location in the absence of the pathogen. Linkage drag and the confounding effects of environmental variation associated with conventional breeding methods would also be eliminated. The ability to map genes contributing towards variation in complex traits with enough accuracy to be of use for plant breeding applications have made possible through the development of comprehensive molecular- marker maps. Loci contributing towards quantitative variation in disease resistance have mapped in tomato for resistance against insects<sup>16</sup> in potato for resistance against cyst-nematodes<sup>11</sup> in pea for resistance against *Ascochyta* blight<sup>5</sup> in potato for resistance against late blight<sup>13</sup> and in maize for resistance against gray leaf spot<sup>3</sup> northern corn leaf blight<sup>6</sup> and stalk and ear rot<sup>17</sup>.

Quantitative trait locus analysis has been used to study the stability of fungal-resistance QTL across environments<sup>21</sup>. The first detailed RFLP marker-based genetic map of pearl millet was published by Liu *et al.*<sup>14</sup> and extended by Devos *et al.*<sup>4</sup> and Qi *et al.*<sup>19</sup>. So that QTL analysis is now possible even in this little-studied crop. QTLs have identified and mapped for DM resistance<sup>2,7,10</sup> and blast resistance<sup>15</sup> drought tolerance and grain yield<sup>22</sup> and for characters involved in domestication<sup>18</sup>. The present study was aimed to identify QTL regions associated with downy mildew resistance using molecular markers.

## MATERIAL AND METHODS

### Plant material

Recombinant Inbred Lines (RILs) developed from the parents: 81B-P13, induced downy mildew resistant selection from Tift23D<sub>2</sub>B, now highly susceptible to downy mildew disease, low grain density for Zn and Fe, dwarf plant height and small grain size was crossed

with AIMP 92901-deriv-P13, Selection from AIMP 92901, downy mildew resistant, high grain density for Zn and Fe, short plant height and small grain size. The crossed seed were selfed to obtain F<sub>7</sub> generation population. 295 RILs were used in the present study.

### Pathogen material

Sg445 from Banaskantha, Gujarat, Sg519 from Rewari, Haryana and Sg526 from Jodhpur, Rajasthan.

### Screens at the ICRISAT

Between 0-40 seeds of each F<sub>7</sub> RIL population were sown 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. Pots were placed in RBD in 3 replications in a glasshouse. When the seedlings were at the coleoptile to one-leaf stage the inoculum was prepared. Leaves from infected plants of 7042 and HB-3 growing in the downy mildew field nursery were wiped free of old sporangia and incubated in covered boxes lined with moist blotting paper. The resulting sporangia were collected into tap water at room temperature and the concentration assessed and adjusted to approximately  $1.0 \times 10^6$  sporangia ml<sup>-1</sup>. Each pot of seedlings was sprayed with approximately 5 ml of inoculum using a hand-pumped sprayer. The pots were covered with polythene sheeting and incubated at 20°C. The percent downy mildew was assessed 14 days later. Screening with each pathotype in 3 replications was carried out at the ICRISAT Asia Centre.

### SSR analysis and Map construction

The polymorphic primers were used for the data analysis with the 295 RIL mapping population. Linkage analysis was carried out using MapMaker/Exp 3.0<sup>12</sup>. Markers were grouped at LOD 3.0. Map distances were calculated using the Haldane mapping function<sup>8</sup>. Most of the markers are common to the pearl millet consensus map<sup>20</sup> were used to designate and orientate linkage groups (LGs).

### Quantitative Trait Locus Analysis

QTL analysis was carried out using Composite Interval Mapping<sup>9</sup> in QTL Cartographer version 2.5<sup>1</sup>. Two hundred and ninety five

lines had both genotypic and phenotypic information and 39 mapped markers were used for QTL analysis.

## RESULTS

### General statistics for screens

Resistant parent AIMP 92901-deriv-P13 was highly resistant against all the pathogen populations, while susceptible parent 81B-P13 was highly susceptible to all the pathogen populations of the present study. Among all screens, the pathogen population from Gujarat (62.64% mean DMI) was most virulent, while that from Rajasthan was the least virulent (24.38% mean DMI) on the mapping population progenies (Table 1). All screens exhibited high operational heritabilities and CV% is ranging from 9.81- 18.68.

**Table 1: Heritabilities and CV of parents**

	DMI%		
	Sg445	Sg519	Sg526
P1	100	100	91.15
P2	3.0	3.2	2.0
Mean	62.64	26.19	24.38
CV(%)	13.023	18.68	9.81
Heritability	0.93	0.97	0.99

### QTLs for downy mildew resistance (DMR)

Composite interval mapping detected four major downy mildew resistance QTLs on LG1, LG3 and LG4.

### Downy Mildew Resistance QTLs for the Gujarat (Sg445) Pathogen Population

Two major DMR QTLs were identified by QTL Cartographer, LG1 and LG3. On LG1, between Xipes0045 and Xipes0127 with a LOD score of 6.3 at phenotypic variation of 51.96 and on LG3 between Xpsmp2070 and Xipes0166 with phenotypic variation of 34.69 at LOD score of 20. The inheritance of these QTLs showed AIMP 92901-deriv-P03 providing the resistance alleles for the QTLs.

### Downy Mildew Resistance QTLs for the Haryana (Sg 519) Pathogen Population

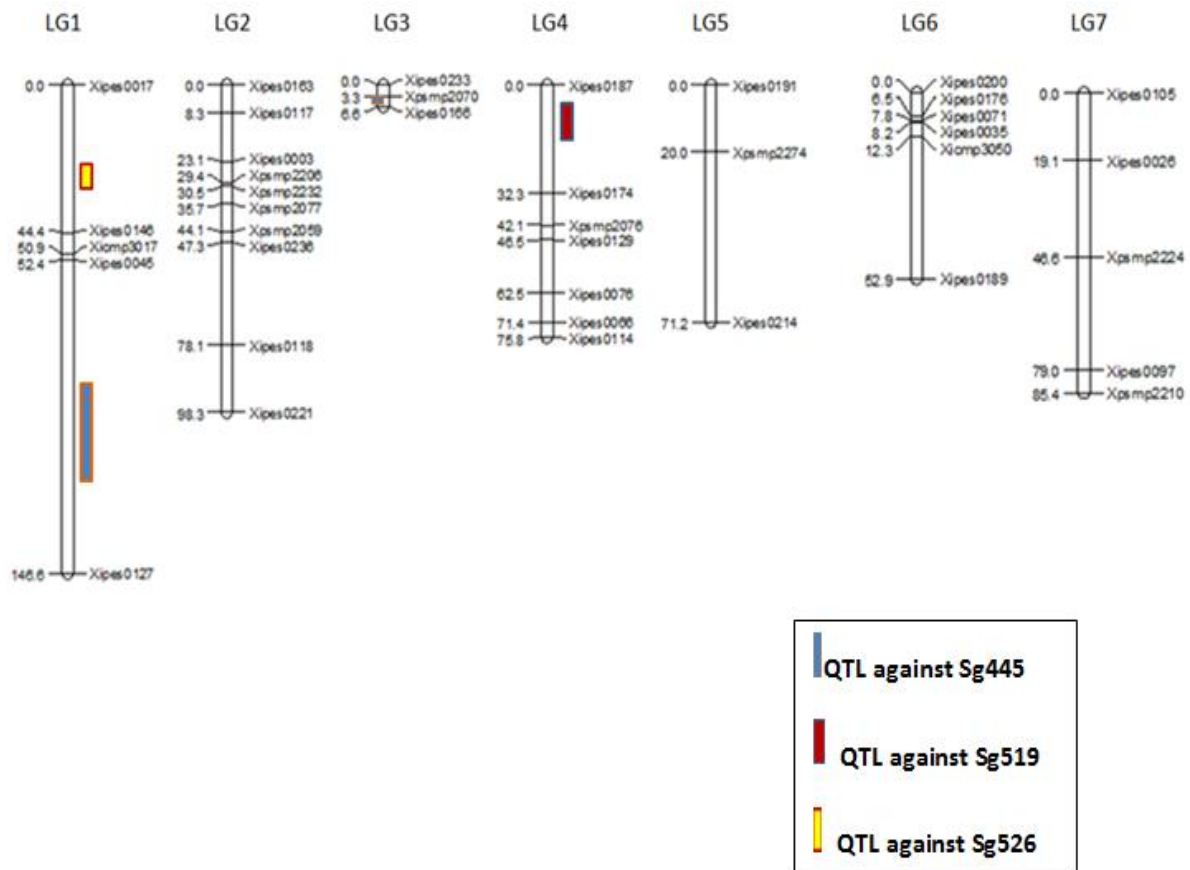
For this pathogen population only one major DMR QTL was discovered. Composite interval mapping method implemented in QTL Cartographer version 2.5 detected one major QTL on LG4 between Xipes0187 and Xipes0174 with a LOD score of 41.0 at phenotypic variation of 75.59. The mode of inheritance of this QTL identified as resistant alleles being contributed by resistant parent AIMP 92901-deriv-P03.

### Downy Mildew Resistance QTLs for the Rajasthan (Sg526) Pathogen Population

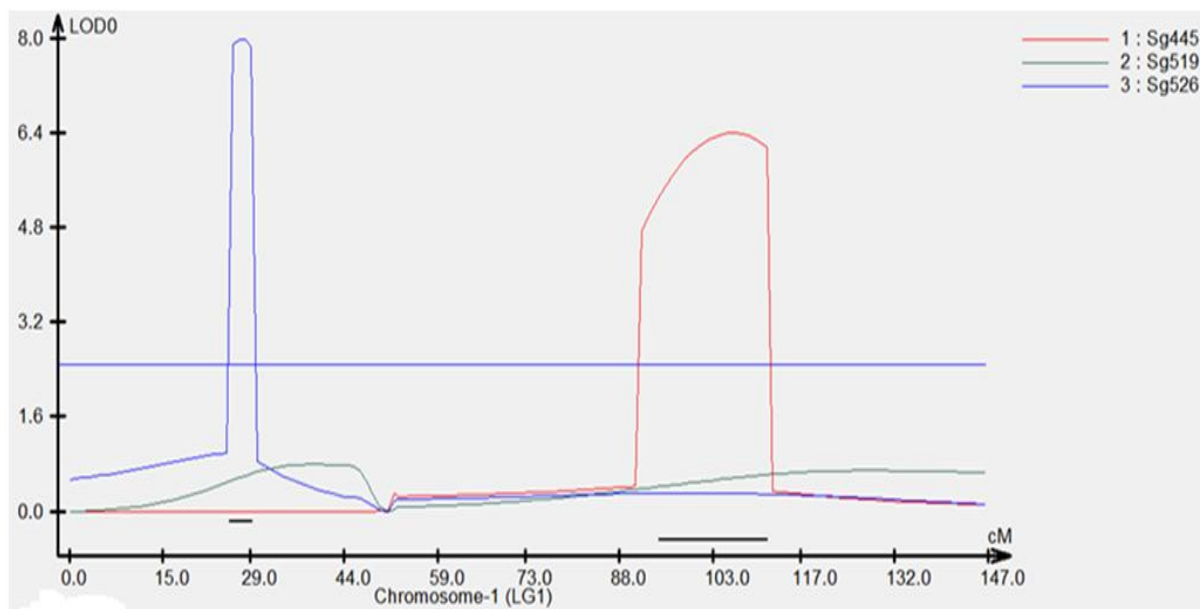
Composite interval mapping using QTL Cartographer detected one major QTL. This QTL on LG1 between Xipes0017 and Xipes0146 with a LOD score of 8.0 at phenotypic variation of 52.05. The inheritance of these QTLs showed AIMP 92901-deriv-P03 providing the resistance alleles for this QTL.

**Table 2: Summary of major pearl millet downy mildew resistance QTLs against three isolates of *S. graminicola* from India in F<sub>7</sub> RIL mapping population of the cross 81B-P13 × AIMP 92901-deriv-P03**

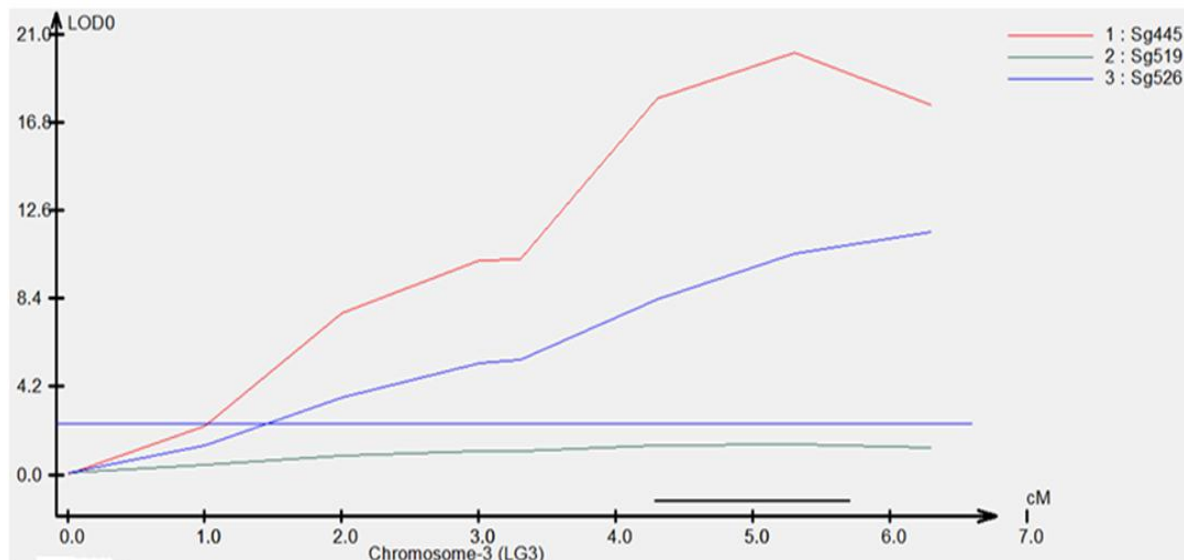
Isolate	Linkage group	Flanking markers	LOD	Variance explained (R <sup>2</sup> )	Additive effect	Inheritance
Sg445	1	Xipes0045- Xipes0127	6.3	51.96%	24.96	Major QTL; AIMP 92901-deriv-P03
	3	Xpsmp2070- Xipes0166	20.0	34.69%	19.56	Major QTL; AIMP 92901-deriv-P03
Sg519	4	Xipes0187- Xipes0174	41.0	75.59%	32.45	Major QTL; AIMP 92901-deriv-P03
Sg526	1	Xipes0017- Xipes0146	8.0	52.05%	27.20	Major QTL; AIMP 92901-deriv-P03



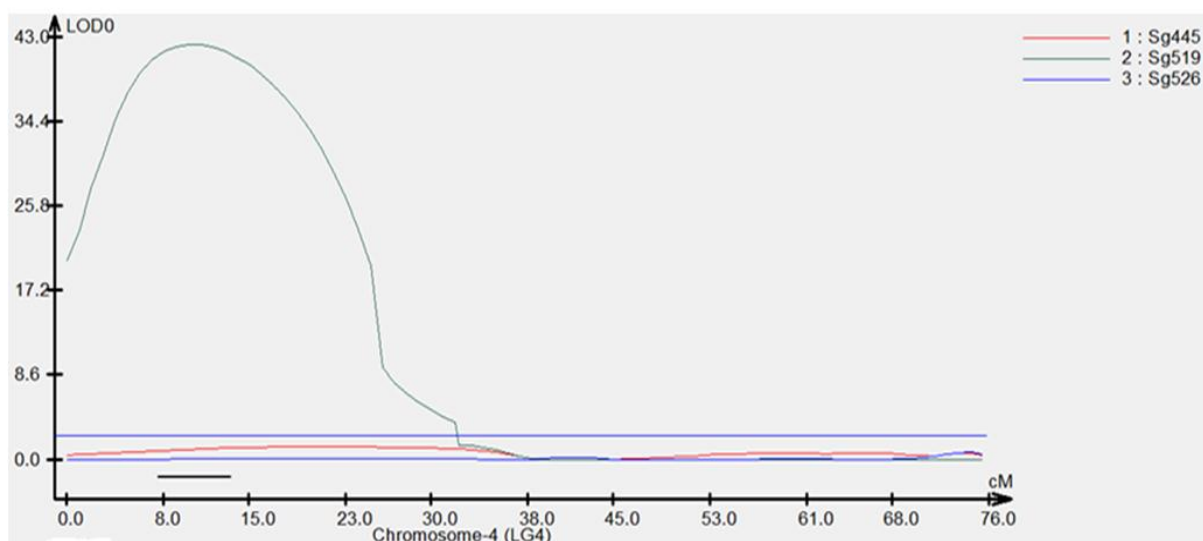
**Fig. 1:** QTL positions on genetic linkage map of pearl millet based on cross 81 B P13 and AIMP 92901-deriv-P03 detected by composite interval mapping on LG (1-7) for resistance to three virulent downy mildew pathogen isolates from India



**Fig. 2:** Comparison of downy mildew resistance QTL LOD peaks obtained for pathogen populations from India mapped on LG1 of the newly constructed genetic linkage map based on cross 81B-P13 and AIMP 92901-deriv-P03 using composite interval mapping method as implemented in QTL Cartographer



**Fig. 3: Comparison of downy mildew resistance QTL LOD peaks obtained for pathogen populations from India mapped on LG3 of the newly constructed genetic linkage map based on cross 81B-P13 and AIMP 92901-deriv-P03 using composite interval mapping method as implemented in QTL Cartographer**



**Fig. 4: Comparison of downy mildew resistance QTL LOD peaks obtained for pathogen populations from India mapped on LG4 of the newly constructed genetic linkage map based on cross 81B-P13 and AIMP 92901-deriv-P03 using composite interval mapping method as implemented in QTL Cartographer**

## DISCUSSION

**DM Screens:** The highly significant variability observed among F<sub>7</sub> RIL populations and high heritabilities demonstrated that a large portion of the variation in DMI among mapping population progenies in this study was attributable to genetic variation, as also reported by Jones *et al*<sup>10</sup>.

QTL mapping for downy mildew resistance (DMR): Four host-plant DMR QTLs were identified in this study. These were distributed across pearl millet linkage groups

of 1,3 and 4. All major DMR QTL was identified for each pathogen population against which the mapping population progenies were screened. DMR QTLs explaining 25-50% of observed phenotypic variation in a particular screen and having high LOD score values were considered major resistance QTLs as suggested by Mackill and Junjian. A summary of these QTLs is presented in Table 2. Among the four QTLs, two QTLs are against Sg445 on linkage groups of 1 and 3, on linkage group

4 QTL position is against Sg 519 and for Sg 526, the QTL is positioned on first linkage group. In the present study there are no common DMR QTLs were identified, but such common blocks of DMR QTLs are of great interest to pearl millet breeders, as they could confer broad-spectrum and durable resistance. Failure to detect these QTLs in this study could be either due to the lack of an appropriate virulence gene to overcome alleles of susceptible parent 81B-P13 or due to presence of a virulence gene in the pathogen population capable of overcoming the resistant allele from resistant parent AIMP 92901- P03.

Mode of inheritance of DMR QTLs: All DMR QTLs identified in the present study, the resistance was inherited from the resistant parent, as previously reported by Jones *et al.*<sup>10</sup>.

#### **FUTURE IMPLICATIONS**

In this study, a pearl millet genetic linkage map of 536.8 cM (Haldane) was constructed with 39 co-dominant marker loci. A total of four different DMR QTLs linked to flanking markers were identified using disease screening data from three downy mildew pathogen populations. Development and mapping of additional polymorphic markers to better saturate gaps in its linkage map would make this pearl millet mapping population ideal for QTL detection.

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