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Development of SCAR marker associated with downy mildew disease resistance in pearl millet (*Pennisetum glaucum* L.)

Sudisha Jogaiah · R. G. Sharathchandra · Niranjan Raj · A. B. Vedamurthy · H. Shekar Shetty

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Abstract Pearl Millet is an important crop coarse grain cereal crop in the semi arid tropics which is extremely susceptible to oomycete plant pathogen *Sclerospora graminicola* causing downy mildew (DM) disease. The aim of the current study is to breed resistance against downy mildew disease into high yielding cultivars of pearl millet. Hence, in the present work a sequence characterized amplified region (SCAR) marker was developed as a molecular screening tool to identify DM resistance source and presented here. Of the 27 inter simple sequence repeats (ISSR) decamer primers used to identify polymorphism amongst pearl millet genotypes ICMR-01007 (P1) and ICMR-01004 (P2) and their populations (F_1 and F_2), only one primer pair ISSR-22 produced polymorphic bands on ICMR-01004 producing 1.4 kb size. The PCR amplification of 1.4 kb band was found tightly linked to the resistant line of ICMR-01004 and also in F_2 segregation population was in the ratio 3:1. This band was cloned, sequenced and candidate SCAR primer ($SCAR_{ISSR\ 863}$) was designed. Segregant analysis of their F_2 progeny revealed that the $SCAR_{ISSR\ 863}$ marker was linked to downy mildew resistance linkage group (χ^2 3:1 = 0.86, $P = 0.22$) with a genetic distance of 0.72 cM. This SCAR marker was further validated using diverse pearl millet lines of India and Africa. Results indicated that the $SCAR_{ISSR\ 863}$ band was amplified in all the seven resistant lines and were absent in five susceptible lines. The confirmation of the ISSR-derived SCAR marker in different genetic backgrounds of pearl millet lines suggests that this marker can be exploited for DM resistance screening in pearl millet breeding programs.

Keywords *Pennisetum glaucum* · *Sclerospora graminicola* · ISSR primer · SCAR marker · Downy mildew resistance

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S. Jogaiah (✉) · A. B. Vedamurthy
Laboratory of Plant and Microbe Interactions, Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad 580 003, India
e-mail: jsudish@gmail.com; jsudish@kud.ac.in

R. G. Sharathchandra
Department of Studies and Research in Environmental Science, Centre for Bioinformation, Tumkur University, Tumkur 572103, India

N. Raj
Department of Microbiology, Karnataka State Open University, Mukthagangotri, Mysore 570006, India

H. S. Shetty
Department of Studies in Biotechnology, Downy Mildew Research Laboratory, University of Mysore, Manasagangotri, Mysore 570006, India

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is reported as the fifth most important cereal crop in the world [1] and stands fourth most important food crop in India, after rice, wheat and sorghum [2]. In India, the crop is grown in an area of 9.3 million hectares with the production of 9 million tons and 957 kg ha^{-1} yield [1, 3]. Pearl millet production is seriously affected by various phytopathogens, of which the major devastating pathogen is the oomycete *Sclerospora graminicola* (Sacc.) Schroet., an obligate biotroph, which causes downy mildew/green ear disease [3, 4]. Since, pearl millet is grown under a wide range of

climatic conditions, it is difficult to manage this disease through cultural practice.

Presently, greenhouse and field screening techniques have been standardized and are used successfully to screen pearl millet lines for downy mildew resistance. However, these techniques are costly, labor intensive and time consuming and their success depends strongly on the environmental conditions. In addition, greenhouse and field screening techniques have limitations like absence of pathogens at the screening sites, low natural disease pressure, environment driven fluctuations in resistance level, age of the plant and stage of development and variability in pathogenicity [5]. Thus, there is a demand for a fast, sensitive, and more reliable screening method for the rapid detection of pearl millet downy mildew resistance genes in the breeding materials. As advances are made in biotechnology and molecular techniques, more rapid and cost effective screening methods have to be employed to enhance the efficiency and success rate of resistance breeding programs. Hence, there is a need to identify marker (s) tightly linked to disease resistance loci that could be used in marker assisted breeding programme in developing high yielding disease-tolerant hybrids.

The conventional breeding way of transferring one or more resistance genes into a variety rely on greenhouse and field screening with different races, which is very difficult, laborious and time consuming process. The introduction of DNA marker technology, pave a way for the development of various types of molecular markers and molecular breeding strategies that have shown great promise in lessening the time and expense for pyramiding resistance genes [6–9]. Molecular markers are now widely used to track loci and genome regions in several crop-breeding programs, as molecular markers tightly linked with a large number of agronomic [10–13] and disease resistance traits [14–16] are available in major crop species.

Until 2000, there were no reports on molecular markers or marker-based genetic linkage map for pearl millet due to which the traits could not be linked to marker locus positions on a non-existent map. By 2001, hundreds of pearl millet molecular markers had been created [17–20], and detailed marker-based genetic linkage maps produced [17, 21]. Such maps could be redirected to study the genomic positions of quantitative trait loci (QTLs) for pearl millet downy mildew resistance [22, 23]. The first molecular marker-based genetic linkage map of pearl millet was constructed by using restriction fragment-length polymorphisms (RFLPs), the marker system of choice then [17]. *Pst*I is a genomic clone from inbred line Tift 23DB, which has now become the base genotype for pearl millet molecular genetics. The development of the pearl millet maps and markers has provided a nucleus around which other millet resources and technologies have been developed. Since the first reports of random amplified polymorphic DNA (RAPD) markers have been published [24], their application has spread rapidly. However, RAPD markers are usually dominant markers, and are sensitive to minor changes in reaction conditions during PCR amplification. Molecular markers facilitate the pyramiding of durable resistance genes into an elite cultivar background within short time and are also less cost-effective [25]. A good molecular marker should produce high polymorphism, co-dominant inheritance, frequent occurrence and even distribution throughout the genome, which will facilitate a easy operation, low cost and high reproducibility that can be successfully utilized in breeding resistant hybrids [26].

In recent years, DNA-based sequenced characterized amplified region (SCAR) markers have been widely used for rapid development of molecular markers for specific traits and pyramiding resistance genes in breeding programme [9, 27]. This method will save money by running only one reaction per plant sample, instead of several reactions. Simple sequence repeats (SSR) marker is one of the most potentially used molecular markers for its great numbers and rich polymorphism [26, 28]. Relative to SSR, SCAR markers that are constructed using inter-SSR (ISSR) primers has the advantages of simple experimental procedure for yielding high polymorphism, easy operation and are more reliable for large-scale marker-assisted selection (MAS) [27, 29].

In our center, screening of pearl millet lines for downy mildew (DM) resistance has been practiced in the All India Coordinating Pearl Millet Improvement Program of Indian council of Agriculture Research to identify markers tightly linked to DM resistance locus which can be utilized in selection of resistant lines and in MAS for gene pyramiding. In this context the aim of this research was to develop the SCAR markers for identification of resistant pearl millet lines and their use in marker assisted selection program.

Materials and methods

Plant materials

Parents ICMR-01007 (P1), ICMR-01004 (P2), F_1, F_2 and F_3 plant generations were used for the present study. ICMR-01007 and ICMR-01004 are derived from downy mildew resistant isolines with the cultivar H 77/833-2 as elite recurrent parent (selection from landrace of the driest region of Haryana, HAU, Hissar) and ICMP 451-P6 donor parent with resistance Quantitative trait loci (QTLs) on linkage group (LG) 1 and 4. ICMR-01007 carries pollinator with LG1 resistance from ICMP 451 in H 77/322-2

background and ICMR-01004 carries pollinator with LG4 resistance from ICMP 451 in H 77/322-2 background [22]. Parents are accessions of ICRISAT, India obtained under a material transfer agreement from university of Mysore and were used throughout the study. F_1 and F_2 seeds are the part of the Ph.D. Research work of R.S. Mahala [30]. The F_3 seeds were generated after selfing the F_2 plants at the experimental plot of Department of Biotechnology, University of Mysore, Mysore.

Pathogen

The DM pathogen, *S. graminicola* highly virulent pathotype-1 which was isolated from the highly susceptible pearl millet cultivar 7042S and maintained on the same cultivar under greenhouse conditions at 22 ± 2 °C with 80 % relative humidity (RH), was used for all inoculation experiments. Downy mildew-infected leaves were collected in evening, washed in running tap water, blotted dry and placed in a moist chamber at 20 °C and >95 % RH for sporulation. Fresh sporangia produced on the leaves were harvested into sterile distilled water, the spore load was adjusted to 4×10^4 zoospores ml^{-1} using a hemocytometer, and used as a source of inoculum in greenhouse studies.

DNA sampling and generation advancement

Seeds of parents ICMR-01007, ICMR-01004 and their crosses, F_1 and F_2 were sown in earthen pots (12–15 cm diameter) containing soil, sand and manure at the ratio of 2:1:1 w/w under greenhouse conditions. Fresh leaf tissues were harvested from 12-day-old plants for DNA extraction. The leaf tissues were frozen in liquid nitrogen and stored at −80 °C until further use.

Screening for downy mildew disease under green house conditions

Parents ICMR-01007 (P1), 1CMR-01004 (P2) and their populations (F_1, F_2 and F_3) were sown in earthen pots containing soil, sand and farm yard manure (FYM) (2:1:1) w/w. Three-day old seedlings were whorl inoculated with the zoospore suspension of *S. Graminicola* (4×10^4 zoospores ml^{-1}). The experiment was carried out in four replicates of 100 seedlings and repeated twice. These pots were arranged in a randomized complete block design and maintained under greenhouse conditions (23 ± 2 °C, 80 % RH).

DNA extraction

One gram of leaf tissue was used for DNA extraction as described in [31].

ISSR–PCR amplification and electrophoresis

Twenty-seven microsatellites repeat primers (ISSR), of which 17 were anchored at 3′ end. Ten unanchored primers were used for PCR amplification (Table S1). Amplification was performed using 1 × PCR buffer, 200 mM of dNTPs, 0.5 µm primer, 0.6 Units of Taq DNA Polymerase (Bangalore Genei, Bangalore, India) and 50 ng of genomic DNA in a final volume of 20 µl for 40 cycles in a thermalcycler (UNO II-BIOMETRA, Gottingen, Germany).

Amplified products were mixed with loading dye (Bromophenol blue, xylene cynol and sucrose), resolved on 1.8 % agarose gel stained with ethidium bromide using 1× TBE buffer pH–8.3 at 60–65 Volts in electrophoresis unit (Maxicell, EC-360 M Apparatus). After the run, the gel was visualized under UV light for scoring the bands.

ISSR–PCR amplification and agarose gel electrophoresis were carried out for three times independently and those fragments that are consistently amplified and reproducible were considered for our study.

Isolation, cloning and sequencing of ISSR fragment

Reproducible polymorphic ISSR bands obtained from cross ICMR-01007 × ICMR-01004 were excised individually from the agarose gel and purified using a QIAGEN gel extraction kit and re-amplified in 20 µl of reaction mixture using the same primer that identified the polymorphic ISSR fragment. The purified polymorphic ISSR fragments were cloned in pGEM-T easy vector (T/A cloning) and sequenced (MWG, Biotech, Bangalore, India).

Design of SCAR primers

Candidate SCAR markers were designed using Primer 3 and primer sequences were analyzed with Net-Primer (Premier Biosoft International, Palo Alto, CA) software. Primer sequences are as follows,

UOM1-Pm-ICMR-01004-F: 5′-AAGGCCGCTCTGCTGCTG-3′
UOM1-Pm-ICMR-01004-R: 5′-TAGTAGTAAGGCGGCGGCG-3′

The primers were synthesized by Sigma Aldrich (Bangalore, India).

PCR amplification of SCAR marker

SCAR marker was amplified with original same DNA (used for ISSR study) to check the reproducibility of the SCAR marker. SCAR-PCR amplification and agarose gel electrophoresis were carried out for three times for parents ICMR-01007, ICMR-01004 and its cross derivatives (F_1)

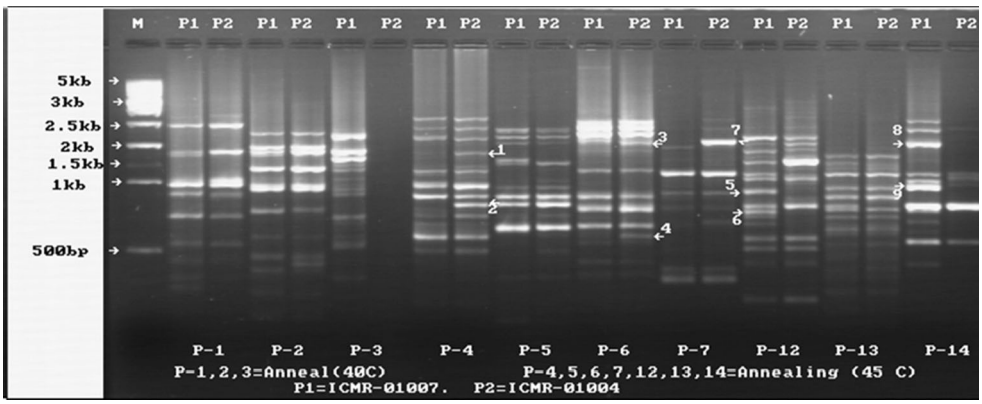

Fig. 1 Polymorphic bands obtained from ISSR primers 4, 6, 7, 13 and 14 on ICMR-01007 and ICMR-01004 pearl millet DM susceptible and resistant lines. No. Of polymorphic bands are pointed with an *arrows*. The lane marked M shows the 5 kb ladder

and 87 F_2 populations. PCR was carried out in 20 µl reaction volume as described above. SCAR PCR was performed for one initial step of 3 min at 94 °C followed by 30 cycles with the temperature profile 45 s at 94 °C, 30 s at 70 °C annealing temperature, 90 s extension at 72 °C and a final extension for 7 min at 72 °C. Reaction products were resolved by electrophoresis unit. PCR reactions with SCAR primers were repeated at least three times. Data was scored for the presence of SCAR marker and recorded in binary form (±).

Validation of SCAR marker

DNA was extracted from 12 diverse pearl millet lines of which 3 genotypes belonging to highly resistant (HR) category viz., P-1449-2, IP-18292 and IP-18293, 4 genotypes which are under resistant (R) category namely, 700651, P7-4, P310-17 and ICMP-451. Genotypes 7042S and NHB-3 were highly susceptible, and the remaining 7042R, HHB67 and 852B genotypes were susceptible to downy mildew disease in India and Africa. PCR reactions and conditions were carried out as described in PCR amplification using SCAR marker.

Linkage analysis

Student's Chi square test was used to analyze the SCAR marker segregation in F_2 and phenotypic segregation in F_3 populations and to determine possible linkages between the ISSR/SCAR marker(s) and the DM resistance loci.

Statistical analysis

PCR reactions with SCAR primer(s) were repeated at least thrice. Data was scored for the presence of SCAR marker and recorded in binary form (±). Goodness-of-fit test ($\chi 2$ analysis) was performed using Microsoft Excel spreadsheet software (Microsoft Corp., Redmond, WA) for the segregation ratio of 3:1 (Resistant: Susceptible) in the F_2 population. SCAR marker segregation, phenotypic segregation and linkage analysis were carried out by using Student's Chi square test.

Results

Screening for downy mildew disease under green house conditions

Parents ICMR-01007 and ICMR-01004 upon pathogen inoculation revealed susceptible and resistant reaction with 15.3 and 5.3 % DM disease incidence, respectively. The F_1 plants of the cross ICMR-01007 × ICMR-01004 recorded highly resistant reaction with 4.7 % disease incidence. Differential downy mildew incidence was recorded in plants of F_3 population selfed from F_2 plants. Among the 87 plants 27 plants had less than 5 % disease, followed by 15 plants with 10 %, 27 plants with 10.1–25 % and 18 plants showed 25 % disease respectively.

ISSR–PCR amplification

Out of the 27 ISSR primers screened, primers ISSR-4, 6, 7, 11, 13, 14, 17, 19, 20 and 22 produced polymorphic bands on ICMR-01007 and ICMR-01004 (Figs. 1, 2). Two primers, ISSR-11 and 14, produced polymorphic bands on ICMR-01007 and one primer ISSR-22 produced polymorphic band on ICMR-01004. On repeated PCR amplification, only primer pair ISSR-22 produced polymorphic band on ICMR-01004 showing 1.4 kb in size. The 1.4 kb band identified from the parent ICMR-01004 was screened for the presence or absence in the parents ICMR-01007, ICMR-01004 and their F_2 populations. The data was recorded in the binary form (±).

Marker data of F_2 and phenotypic data of F_3 were correlated. The 1.4 kb band was found tightly linked to the resistant line ICMR-01004 and also in F_2 segregation population in the ratio 3:1 (Fig. 3a–d) indicating the

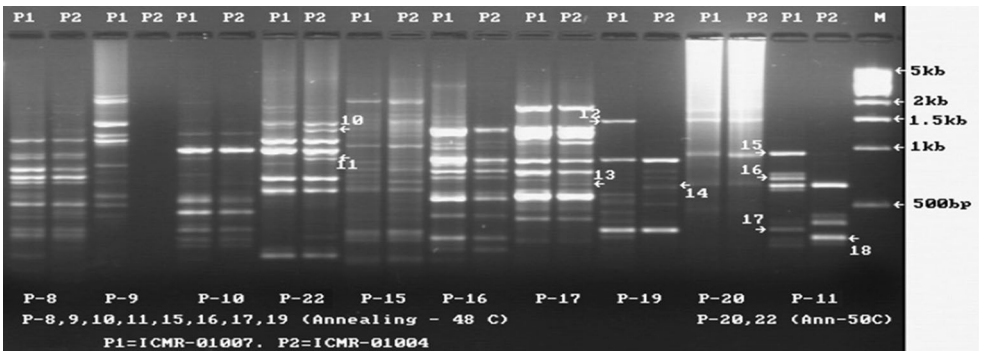

Fig. 2 Polymorphic bands obtained from ISSR primers 22, 17, 19, 20 and 11 on ICMR-01007 and ICMR-01004 pearl millet DM susceptible and resistant lines. No. of polymorphic bands are pointed with an *arrows*. The lane marked M shows the 5 kb ladder

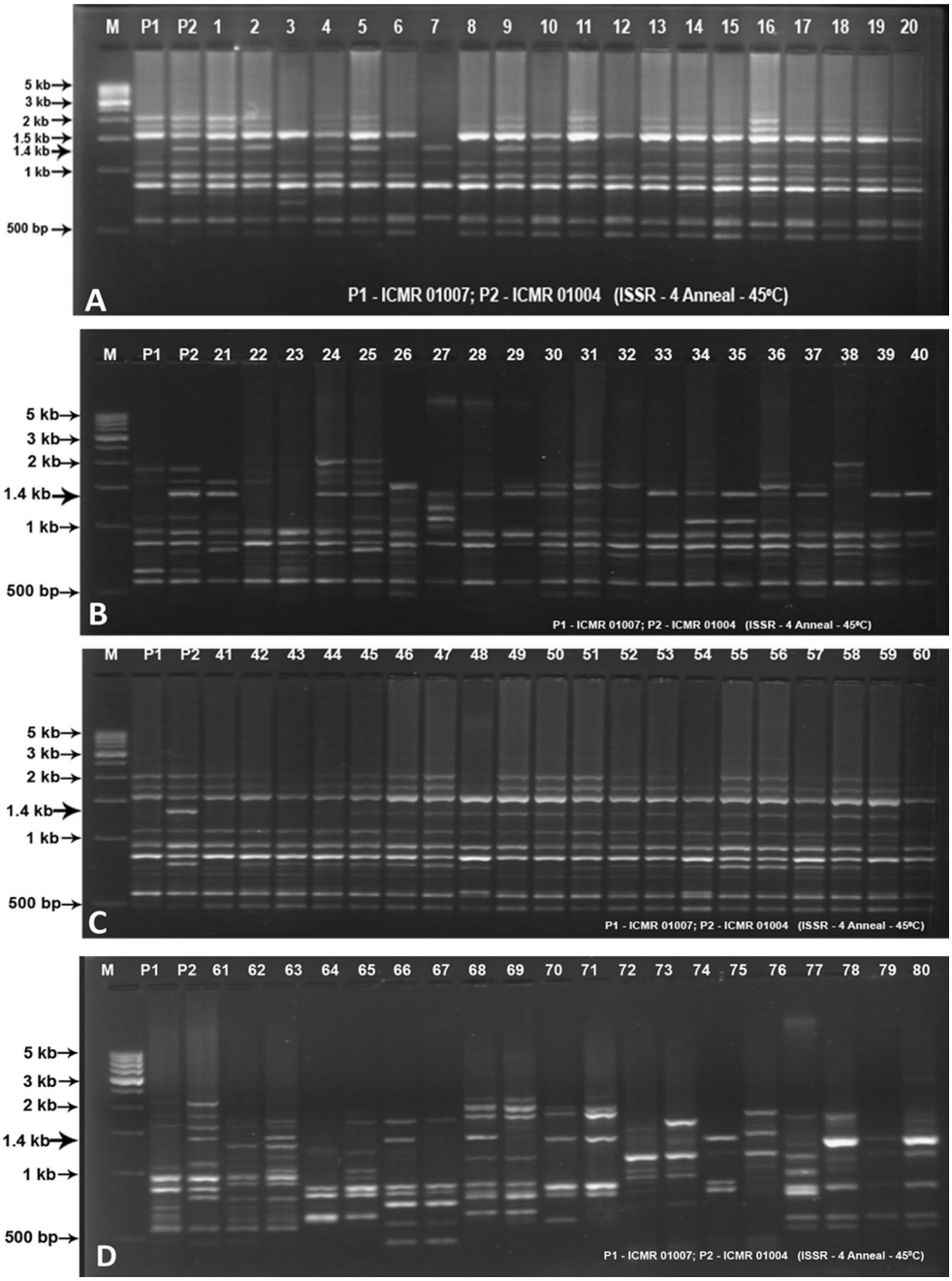

Fig. 3 **a–d** Agarose gel (1.8 %) stained by ethidium bromide showing amplification of parents ICMR-01007 (P1), ICMR-01004 (P2) and in F_2 populations **a** (01–20); **b** (21–40); **c** (41–60); **d** (61–80) using primer pair $(CCTA)_4$. Lane marked M shows the 5 kb ladder

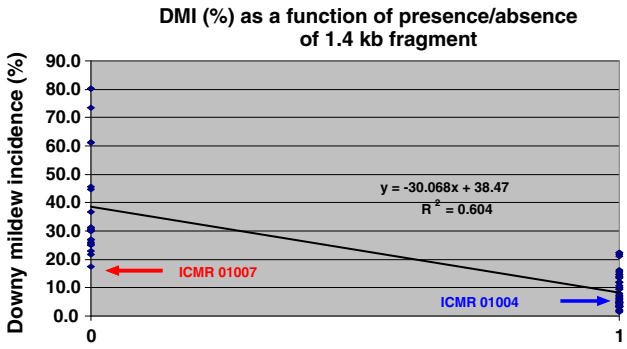

Fig. 4 Association of 1.4 kb band from ICMR-01004 with F_2 marker data and phenotypic data of F_3

presence of the resistant gene in this line is associated with LG4 of pearl millet. The dominant marker allele identified in this study from both the resistant (ICMR-01004) and susceptible parent (ICMR-01007) was confirmed by marker and phenotypic data of F_2 and F_3 populations respectively (Fig. 4).

The product 1.4 kb was cloned. Sequences were analyzed for sequence homology in National Center for Biotechnology Information (NCBI) GenBank, the sequence data of all clones showed same sequence. Sequence homologies were analyzed with the BLASTN and BLASTX programs (1) at the NCBI network service. Sequences have been deposited in the GenBank (NCBI accession no EU729336).

SCAR primer design and amplification specificity

Candidate SCAR primers were designed based on sequence data of EU729336 using Primer 3 service package. PCR amplification using SCAR primer pairs (UOM1-PM–ICMR-04-F/R) from ISSR-22 detected a single band of 863 bp band in ICMR-01004, which is absent in ICMR-01007. Upon screening the $SCAR_{ISSR\ 863}$ primer pairs with 87 plants of F_2 segregation population (Fig. 5a–c), $SCAR_{ISSR\ 863}$ band was present in 58 lines and absent in 29 lines (Table 1).

Validation of SCAR marker

The $SCAR_{ISSR\ 863}$ marker was found to be present in the diverse lines having highly resistant and resistant reaction to DM disease viz., P-1449-2 (HR), IP-18292 (HR), IP-18293 (HR), 700651 (R), P7-4 (R), P310-17 (R) and ICMP-451 (R), and absent in susceptible and highly susceptible diverse lines viz., 7042S (HS), NHB-3 (HS), 7042R (S), HHB67 (S), and 852B (S) (Fig. 6).

Linkage analysis

An F_2 population of the cross ICMR-01007 × ICMR-01004 was analyzed for segregation of marker and F_3 populations with the downy mildew resistance phenotype. In the F_2 population, the resistant $SCAR_{ISSR\ 863}$ marker and F_3 resistant phenotypes were dominant and segregated as 3:1 (χ^2 3:1 = 0.86, $P = 0.22$) with a genetic distance of 0.72 cM (Table 2a, b).

Discussion

During selection, a breeder is prone to determine the presence of a specific disease resistance gene(s). A DNA marker for the gene of interest can greatly facilitate plant breeding procedure and further eliminate the need for phenotypic expression of the gene.

The parent ICMR-01004 carrying resistant QTLs on LG4 with ICMP-451-P6 background confer high degree of DM resistance under Indian and African conditions [22]. In this study, the DM disease screening data of F_1 plants remained highly resistant corresponding to its parental line ICMR-01004 indicating dominance of resistance. Also, the fifty-eight plants of F_2 provided resistant reaction and 29 plants categorized under susceptible showing a monogenic segregation ratio (3:1, expected value = 65.25: 21.75). Further, the DM disease data of the F_3 population selfed from F_2 plants revealed that out of the 87 plants, 42 were resistant and 45 remained susceptible.

To develop molecular markers, we used 27 ISSR primers and studied the linkage analysis among parents and its progeny. Primer pair ISSR-22 detected a polymorphic fragment of 1.4 kb in both the parents and the F_2 populations. The 1.4 kb band was cloned, sequenced, catalogued and its resistance linkage on pearl millet LG4 was established by converting the DM resistant candidate $SCAR_{ISSR\ 863}$ marker based on sequence data of 1.4 kb (EU729336). We also correlated the $SCAR_{ISSR\ 863}$ marker data obtained from F_2 plants with that of DM disease data of F_3 plants. The result from the current finding, in which $SCAR_{ISSR\ 863}$ band was present in 47 of the 58 resistant F_2 plants, but absent in all of the 29 susceptible F_2 plants, confirms the tight linkage of the $SCAR_{ISSR\ 863}$ marker to pearl millet LG4 and indicates that this marker may be useful for MAS. This frequency of finding a marker associated to plant disease resistance was comparable to other studies using ISSR-derived SCAR marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines, ISSR-SCAR for conferring resistance against wheat leaf rust [32], ISSR-derived SCAR markers linked to the Mungbean yellow mosaic virus (MYMV) resistance gene [29]. SCAR markers linked to the *Citrus tristeza* virus resistance gene from *Poncirus trifoliate* has been reported by

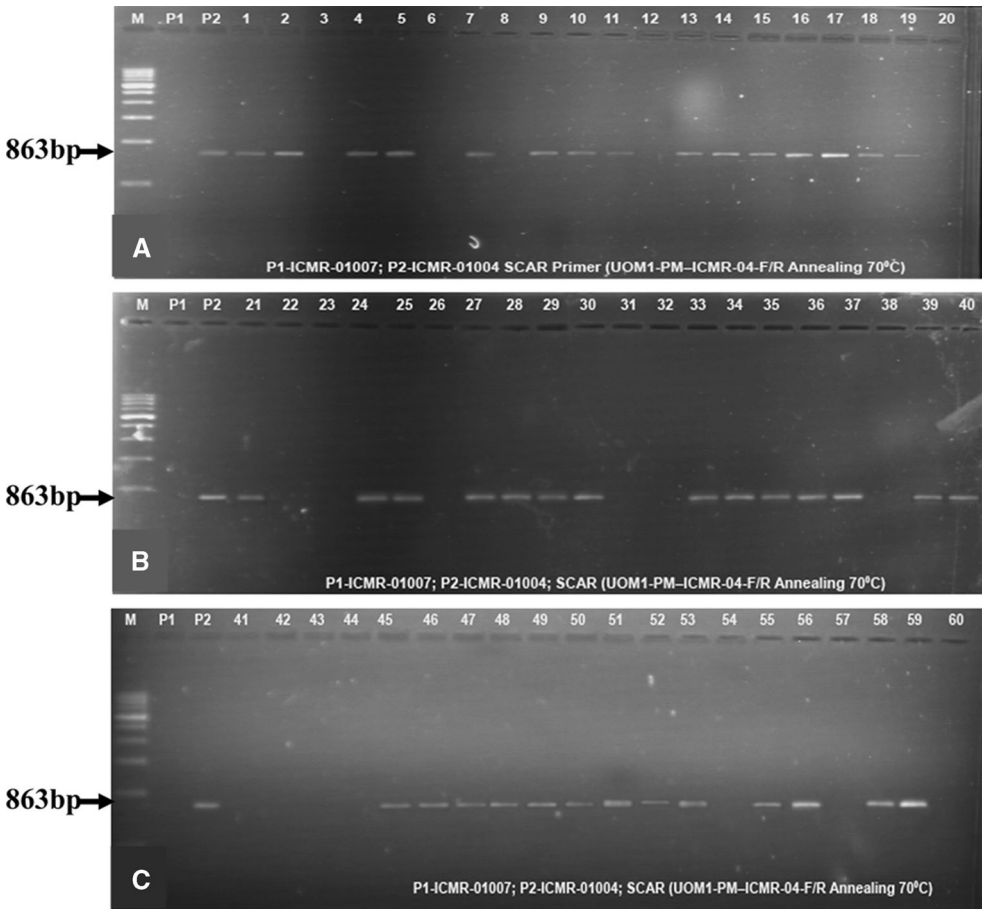

Fig. 5 **a–c:** Candidate SCAR primers designed based on sequence data (NCBI accession no EU729336). PCR amplification using SCAR primer pairs (UOM1-PM–ICMR-04-F/R) showing presence of 863 bp band in ICMR-01004 (P2) and absent in ICMR-01007 (P1) and is inherited in 3:1 (R:S) segregation ratio in F_2 progenies is associated to downy mildew resistant gene. **a** (1–20); **b** (21–40); **c** (41–60). Lane marked M shows the 5 kb ladder

Table 1 4 × 2 contingency Chi square

Category	Observed		Expected (assuming no linkage)		(o−e)^2/e
	Present	Absent	Present	Absent	
HR	27	0	20.25	6.75	9.000
R	15	0	11.25	3.75	5.000
S	16	11	20.25	6.75	3.568
HS	0	18	13.50	4.50	54.000
Total	58	29	62.25	21.75	71.568*
			0.81	2.42	
Marker Chi Square			3.22		
Disease (HR + HR + R)			0.22	0.65	
Chi Square			0.86**		

* 71.568 Highly significant departure from expected distribution if there is no association

** 0.860 Significant Chi square for goodness of fit to 3:1 ratio

[33]. The development of a SCAR marker (*RYSC3*) based on nucleotide differences within resistance gene like fragments isolated from a potato plant carrying the *Ryadg* gene that confer resistance to potato virus Y disease was reported by [34]. The authors of the present study group successfully developed a ISSR based SCAR marker of 284 bp linked to downy mildew pathotype-1 and has been used in detecting the pathotype-1 strain of pearl millet in ICAR program of India [4]. Earlier reports support the finding of the current study, SCAR marker of 350 bp was developed for detection and differentiation of pathogenic Pierce's disease strains of *X. Fastidiosa* [35]. Species-specific ISSR-derived SCAR marker was designed for the rapid identification of *Sinocalycanthus chinensis* an endangered plant in china [36]. This strategy has been widely and successfully used to develop markers for various traits in a number of crops, for instance, ISSR-derived SCAR markers was successfully designed for identification of the seasonal flowering locus in *Fragaria vesca* [37], SCAR markers linked to the *Tm-1* locus in tomato was designed [38]. SCAR and CAPS markers linked to the beta gene in tomato were developed and utilized in breeding program [39].

The validation of this $SCAR_{ISSR\ 863}$ marker was further assessed using diverse pearl millet lines that are known to be sources of resistance and susceptible varieties for downy mildew disease across India and Africa. The $SCAR_{ISSR\ 863}$ band was amplified in all the seven diverse resistant lines and were absent in five diverse susceptible lines. In support to our findings, several reports in the recent past have described the

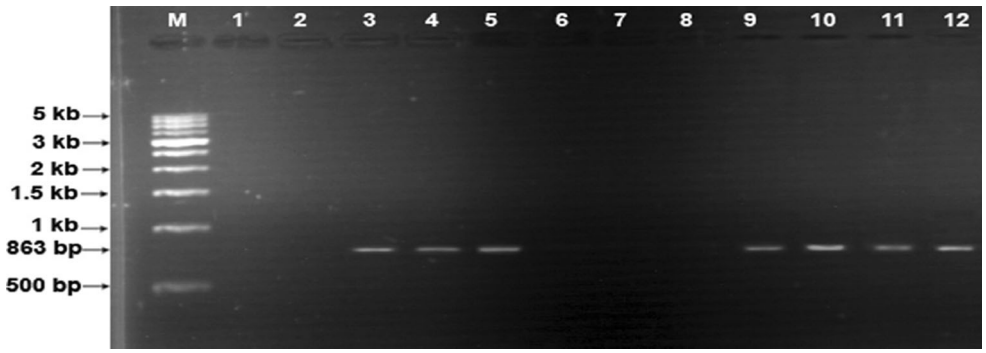

Fig. 6 Detection of SCAR marker in diverse downy mildew resistant and susceptible inbred lines. Presence of 863 bp band in resistant (lanes 3 (P-1449-2), 4, 5, 9, 10, 11 and 12) and absence of the band in susceptible (lanes 1, 2, 6, 7 and 8) inbred pearl millet lines. Lane M: 500 bp ladder,Lanes 1–12 are amplified with $SCAR_{ISSR\ 863}$ marker to genomic DNA of pearl millet inbred lines namely 7042S (HS), NHB-3 (HS), P-1449-2 (HR), IP-18292 (HR), IP-18293 (HR), HHB67 (S), 7042R (S), 852B (S) 700651 (R), P7-4 (R), P310-17 (R) and ICMP-451 (R), respectively

Table 2 a: Genetic distance Linkage analysis of SCAR marker, b: Genetic distance Linkage analysis of SCAR marker

SCAR marker	Observed			Expected values	
	R-1	R-0	Total	Present	Absent
$SCAR_{ISSR\ 863}$-1 (present)	52	6	58	35.33	22.67
$SCAR_{ISSR\ 863}$-0 (absent)	1	28	29	17.67	11.33
Total	53	34	87	53.00	34.00

Genetic distance R (cM)	T1 52/(3 + r)	T2 28/(1−r)	T3 7*(1−r)/[r*(2−r)]	T3-T1-T2
0.03	17.16	28.87	114.89	68.86
0.04	17.11	29.17	85.71	39.44
0.05	17.05	29.47	68.21	21.68
0.06	16.99	29.79	56.53	9.75
0.07	16.94	30.11	48.19	1.14
0.075	16.91	30.27	44.85	−2.33
0.072	16.93	30.17	46.80	−0.30
0.0715	16.93	30.16	47.14	0.05

R-1 represents the presence of DM resistant $SCAR_{ISSR\ 863}$ band. R-0 refers to the absence of $SCAR_{ISSR\ 863}$ band

analysis and validation of resistance genes in other crop species [40–44]. The presence of the specific DM resistant $SCAR_{ISSR\ 863}$ marker in ICMP-451 strongly confirms the specificity of our developed $SCAR_{ISSR\ 863}$ marker with the resistant QTLs present on LG4 of pearl millet. The confirmation of the ISSR-derived SCAR marker in different pearl millet varieties suggested that this marker can be exploited in a wide range of genetic backgrounds. A necessary pre-requisite for a successful breeding program is isolation of a gene linked to resistance loci for use of markers in molecular screening. In conclusion identified our $SCAR_{ISSR\ 863}$ marker is expected to facilitate development of DM-resistant pearl millet cultivars carrying the relevant gene for the resistant trait and will be useful for map-based cloning of DM resistance in pearl millet which could effectively be used as a low cost high-throughput alternative for conventional breeding program to produce DM resistant high yielding pearl millet variety/hybrid.

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Conflict of interest The authors have no conflict of interest related to the work described in this manuscript.

References

1. Khairwal IS (2008) Pearl millet coordinators review report. Indian Council of Agricultural Research, Mandor, pp 1–23
2. Sudisha J, Anand Kumar S, Thakur RP, Rao VP, Shekar Shetty H (2009) Molecular characterization of *Sclerospora graminicola*, the incitant of pearl millet downy mildew revealed by ISSR markers. J Phytopathol 157:748–755
3. Sudisha J, Kumar Arun, Amruthesh KN, Niranjana SR, Shekar Shetty H (2011) Elicitation of resistance and defense related enzymes by raw cow milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. Crop Prot 30:794–801
4. Sudisha J, Ananda Kumar S, Shetty NP, Shekar Shetty H (2009) Cloning and development of pathotype specific SCAR Marker associated to *Sclerospora graminicola* isolates from pearl millet. Aust J Plant Pathol 38:216–221
5. Pushpalatha HG, Sudisha J, Ashok S, Geetha NP, Kini KR, Shekar Shetty H (2013) Association between accumulation of allene oxide synthase activity and development of resistance against downy mildew disease of pearl millet. Mol Biol Rep 40:6821–6829
6. Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 363:557–572
7. Varshney RK, Mahendari T, Agarwal RK, Borner A (2007) Genic molecular markers in plants. Development and Applications. In: Varshney RK, Tuberosa R (eds) Genomics-Assisted Crop Improvement. Genomics Approaches and Platforms, vol 1. Springer, The Netherlands, pp 13–29
8. Delannay X, McLaren G, Ribaut J-M (2012) Fostering molecular breeding in developing countries. Mol Breed 29:857–873
9. Burdon JJ, Barrett LG, Rebetzke G, Thrall PH (2014) Guiding deployment of resistance in cereals using evolutionary principles. Evol Appl 7:609–624
10. Xiao B, Huang Y, Tang N, Xiong L (2007) Over-expression of a *LEA* gene in rice improves drought resistance under the field conditions. Theor Appl Genet 115:35–46
11. Wu J, Zhuang W, Xiong Y, Yang Y, Deng H, Deng Q (2010) Breeding of new hybrid rice combination Y Liangyou 7 with high yield and good quality by introducing yield-increase QTLs of wild rice. Hybrid Rice 25:20–22
12. Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet 44:950–954
13. Rao Y, Yang Y, Huang L, Pan J, Ma B, Qian Q, Zeng D (2013) Research progress on cold stress in rice. Mol Plant Breed 11:443–450
14. Yang Q, Lin F, Wang L, Pan Q (2009) Identification and mapping of *Pi41*, a major gene conferring resistance to rice blast in the *Oryza sativa subsp indica* reference cultivar, 93-11. Theor Appl Genet 118:1027–1034
15. Hua L, Wu J, Chen C, Wu W, He X, Lin F, Wang L, Ashikawa I, Matsumoto T, Wang L, Pan Q (2012) The isolation of *Pi1*, an allele at the *Pik* locus which confers broad spectrum resistance to rice blast. Theor Appl Genet 125:1047–1055
16. Miah M, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Asfaliza R, Latif MA (2013) Blast resistance in rice: a review of conventional breeding to molecular approaches. Mol Biol Rep 40:2369–2388
17. Liu CJ, Witcombe JR, Pittaway TS, Nash M, Busso CS, Hash CT, Gale MD (1994) An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481–487
18. Allouis S, Qi X, Lindup S, Gale MD, Devos KM (2001) Construction of a BAC library of pearl millet, *Pennisetum glaucum*. Theor Appl Genet 102:1200–1205
19. Qi X, Lindup S, Pittaway TS, Allouis S, Gale MD, Devos KM (2001) Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. Biotechniques 31:355–362
20. Hash CT, Witcombe JR (2001) Pearl millet molecular marker research. Int Sorghum Millets Newsl 42:8–15
21. Devos KM, Pittaway TS, Reynolds A, Gale MD (2000) Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. Theor Appl Genet 100:190–198
22. Breese WA, Hash CT, Devos KM, Howarth CJ (2002) Pearl millet genomics—an overview with respect to breeding for resistance to downy mildew. In: Leslie JF (ed) Sorghum and Millets Pathology 2000. Iowa State Press, Ames, pp 243–246
23. Jones ES, Breese WA, Liu CJ, Singh SD, Shaw DS, Witcombe JR (2002) Mapping quantitative trait loci for resistance to downy mildew in pearl millet: field and glasshouse screens detect the same QTL. Crop Sci 42:1316–1323
24. Williams JGK, Kubelik AR, Livak KL, Rafalski JA, Tingey SV (1990) DNA polymorphisma amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:653–659
25. Djian-Caporalino C, Palloix A, Fazari A, Marteu N, Barbary A, Abad P, Sage-Palloix AM, Mateille T, Risso S, Lanza R, Taussig C, Castagnone-Sereno P (2014) Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. BMC Plant Biol 14:53
26. Li SJ, Xie HW, Qian MJ, Chen GH, Li SQ, Zhu YG (2012) A Set of SCAR Markers Efficiently Differentiating Hybrid Rice. Rice Sci 19:14–20
27. Dhole VJ, Reddy KS (2013) Development of a SCAR marker linked with a MYMV resistance gene in mungbean (*Vigna radiata* L. Wilczek). Plant Breed 132:127–132
28. Wei X, Wang L, Zhang Y, Qi X, Wang X, Ding X, Zhang J, Zhang X (2014) Development of Simple Sequence Repeat (SSR) Markers of Sesame (*Sesamum indicum*) from a Genome Survey. Molecules 19:5150–5162
29. Souframanien J, Gopalakrishna T (2006) ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [*Vigna mungo* (L.) Hepper]. Plant Breed 125:619–622
30. Mahala RS (2010) Resistance gene deployment using marker assisted selection in developing pearl millet hybrids resistant to downy mildew disease. PhD Thesis, University of Mysore
31. Bhattacharjee R, Bramel PJ, Hash CT, Kolesnikova-Allen MA, Khairwal IS (2002) Assessment of genetic diversity within and between pearl millet landraces. Theor Appl Genet 105:666–673
32. Li-Rong Z, Da-Qing X, Wen-Xiang Y, Da-Qun L (2004) A molecular marker of leaf rust resistance gene *Lr37* in wheat. Chin J Agric Biotechnol 1:139–142
33. Deng Z, Huang S, Xiao S, Gmitter FG (1997) Development and characterization of SCAR markers linked to the *Citrus tristeza* virus resistance gene from *Poncirus trifoliata*. Genome 40:697–704
34. Kassi K, Morikawa Y, Sorri VA, Valkonen JPT, Gebhardt C, Watanabe KN (2000) Development of SCAR markers to the PVY resistance gene *Ryadg* based on a common feature of plant disease resistance genes. Genome 43:1–8
35. Travensolo RF, Ciapina LP, Lemos EGM (2005) Development of a scar marker for Pierce's Disease strains of *Xylella fastidiosa*. *Fitopatol. bras.* [online]. 30:115-120
36. Ye Q, Qiu YX, Quo YQ, Chen JX, Yang SZ, Zhao MS, Fu CX (2006) Species-specific SCAR markers for authentication of *Sinocalycanthus chinensis*. J Zhejiang Univ Sci B 7:868–872
37. Albani MC, Battey NH, Wilkinson MJ (2004) The development of ISSR-derived SCAR markers around the seasonal flowering locus (*SFL*) in *Fragaria vesca*. Theor Appl Genet 109:571–579

38. Ohmori T, Murata M, Motoyoshi F (1996) Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato. Theor Appl Genet 92:151–156
39. Zhang Y, Stommel JR (2001) Development of SCAR and CAPS markers linked to the beta gene in tomato. Crop Sci 41:1602–1608
40. Sowmini Jayamani (2014) Validation of Molecular Markers Linked with Yellow Mosaic Disease Resistance in Blackgram *Vigna mungo* (L.) Hepper. Legume Genomics and Genet 5:25–30
41. Namayanja A, Buruchara R, Mahuku G, Rubaihayo P, Kimani P, Mayanja S, Eyedu H (2006) Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. Euphytica 151:361–369
42. Fondevilla S, Rubiales D, Moreno MT, Torres AM (2008) Identification and validation of RAPD and SCAR markers linked to the gene *Er3* conferring resistance to *Erysiphe pisi* DC in pea. Mol Breed 22:193–200
43. Patil RM, Manoj D, Oak MD, Shubhada A, Tamhankar SA, Sourdille P, Rao VS (2008) Mapping and validation of a major QTL for yellow pigment content on 7AL in durum wheat (*Triticum turgidum* L. ssp. *durum*). Mol Breed 21:485–496
44. Grewal TS, Rossnagel BG, Scoles GJ (2008) Validation of molecular markers for covered smut resistance and marker-assisted introgression of loose and covered smut resistance into hulless barley. Mol Breed 21:37–48