



Mapping quantitative trait loci (QTLs) associated with resistance to major pathotype-isolates of pearl millet downy mildew pathogen

Durgaraju Chelpuri · Rajan Sharma · Kilaru Kanaka Durga · Pooja Katiyar · Mahesh D. Mahendrakar · Ram Baran Singh · Rattan S. Yadav · Rajeev Gupta · Rakesh K. Srivastava

Accepted: 7 March 2019

© Koninklijke Nederlandse Planteziektenkundige Vereniging 2019

Abstract Downy mildew (DM) caused by *Sclerospora graminicola* is the most devastating disease of pearl millet. It may lead to annual grain yield losses of up to ~80% and substantial deterioration of forage quality and production. The present study reports construction of the linkage map integrating simple sequence repeat (SSR) markers, for detection of quantitative trait loci (QTLs) associated with DM resistance in pearl millet. A mapping population comprising of 187 F₈ recombinant inbred lines (RILs) was developed from the cross (ICMB 89111-P6 × ICMB 90111-P6). The RILs were evaluated for disease reaction at a juvenile stage in the greenhouse trials. Genotyping data was generated from 88 SSR markers on RILs and used to construct genetic linkage map comprising of 53 loci on seven linkage groups (LGs) spanning a total length of 903.8 cM with an average adjacent marker distance of 18.1 cM. Linkage group 1 (LG1; 241.1 cM) was found to be longest and

LG3 the shortest (23.0 cM) in length. The constructed linkage map was used to detect five large effect QTLs for resistance to three different pathotype-isolates of *S. graminicola* from Gujarat (Sg445), Haryana (Sg519) and Rajasthan (Sg526) states of India. One QTL was detected for isolate Sg445 resistance, and two each for Sg519 and Sg526 resistance on LG4 with LOD scores ranging from 5.1 to 16.0, explaining a wide range (16.7% to 78.0%) of the phenotypic variation (R^2). All the five co-localized QTLs on LG4 associated with the DM resistance to the three pathotype-isolates were contributed by the resistant parent ICMB 90111-P6. The QTLs reported here may be useful for the breeding programs aiming to develop DM resistant pearl millet cultivars with other desirable traits using genomic selection (GS) approaches.

Keywords Quantitative trait loci (QTLs) · Downy mildew · Recombinant inbred lines (RILs) · Linkage map · Linkage groups (LG) and simple sequence repeats (SSRs)

Abbreviations

CIM	Composite interval mapping
cM	Centimorgan
DM	Downy mildew
DMI	Downy mildew incidence
DMR	Downy mildew resistance
FAO	Food and Agriculture Organization of the United Nations
LG	Linkage group

D. Chelpuri · R. Sharma · P. Katiyar · M. D. Mahendrakar · R. B. Singh · R. Gupta · R. K. Srivastava (✉)
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Telangana State 502 324, India
e-mail: r.k.srivastava@cgiar.org

D. Chelpuri · K. K. Durga
Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad, Telangana State 500 030, India

R. S. Yadav
Crop Genetics, Genomics and Breeding Division, Aberystwyth University, Aberystwyth SY23, UK

LOD	Logarithm of the odds
MIM	Multiple interval mapping
QTLs	Quantitative trait locus or quantitative loci, relying on context
RILs	Recombinant inbred lines
RH	Relative humidity
SSRs	Simple sequence repeats

Introduction

Pearl millet [*Pennisetum glaucum* (L) R. Br.] ($2n = 2x = 14$) is an important C_4 small-grained field crop of marginal farming systems which possesses an abundance of nutrients, and has low glycemic index (GI). It is predominantly grown and consumed by subsistence and poor people in tropical and sub-tropical regions of the world (Kumar et al. 2017). Pearl millet belongs to the grass family *Poaceae* and sub-family *Panicoideae* was domesticated over 4,500 years ago from *Pennisetum glaucum* ssp. *monodii* (Brunken 1977; Manning et al. 2011). Pearl millet is a staple cereal crop cultivated on approx 28 million ha in adverse agro-climates of the tropical and sub-tropical regions globally where other cereal crops (*Oryza sativa*, *Triticum aestivum*, *Zea mays*, and even *Sorghum bicolor*) are unlikely to produce projected economic yields (Shivhare and Lata 2017). Pearl millet is well adapted to the areas with scanty rain, dryland, and extreme high temperature and suitable for conservation of moisture content through mulch technique in low-input arid zones.

Pearl millet grains are staple food for millions of the people inhabiting in marginal agricultural production environments of arid and semi-arid areas of the world. Pearl millet is extensively cultivated for food grain, feed, and fodder for livestock, hay, silage, bird feed, building material, and fuel in harsher environments of Sub-Saharan Africa, South-Asia and India that are habitat to hundreds of millions of human beings (Vadez et al. 2012). Pearl millet is naturally blessed with several nutritional properties in comparison to other staple cereals. Its grains are a nutrient-rich and affordable source of carbohydrate, proteins, vitamins, fibers, α -amylase, amino acids, and minerals for providing sustainable food security (Kumar et al. 2016; Anuradha et al. 2017).

Pearl millet is a highly tillering, cross-pollinated, tropically-adapted cereal crop plant with excellent photosynthetic efficiency and biomass production

potential (Sanou et al. 2012). The genome size of cultivated pearl millet is about 1.79 Gb which contains an estimated 38,579 genes (Varshney et al. 2017). Generally, it is preferred as an experimental millet grass for various genetic investigations owing to its diploid chromosome number ($2n = 2x = 14$), short life cycle (60–90 days) and protogynous flowering responsible for controlling unwilling cross-pollination. Pearl millet is majorly cultivated in Rajasthan, Gujarat, Haryana, Maharashtra, Uttar Pradesh, Karnataka, and Andhra Pradesh states of India with a total land of 7.20 million hectares with a production of 8.74 million tons and the national average productivity of 1214 kg ha⁻¹ (DES 2012–13). Pearl millet substantially contributes to the rural economy as most of the dry land farmers rely solely on millet crops and thus millet produce has become a lifeline of resource-poor people.

Moreover, pearl millet productivity has been hampered owing to several constraints and has not been consistent since last more than two decades, ranging from 464 to 768 kg ha⁻¹ (FAOSTAT 2017). Therefore, to maintain pearl millet grain yield in a steady state, developing F₁ hybrids, which usually have relatively high rates of productivity then the open-pollinated crop can be recommended as one of the plausible substitutes. The hybrid cultivars show high levels of vulnerability to abiotic and biotic stresses owing to their narrow genetic base. Besides many of the abiotic (soil infertility, drought, and extreme temperature) and biotic (multiple disease pathogens and pests incidents) productions constraints downy mildew disease is the major biotic constraint to crop productivity of pearl millet, which leads to grain and fodder yield instability (Sharma et al. 2015).

Downy mildew (DM), popularly known as “green ear” disease caused by systemic infection of obligate biotrophic pathogen *Sclerospora graminicola* [(Sacc.) Schroet] of family oomycetes. *S. graminicola* occurs destructively wherever pearl millet is grown (Singh et al. 1993). Downy mildew is the most damaging disease of pearl millet which has been a persistent and serious threat in the millets-growing regions of the tropical and sub-tropical parts of the world (Sharma et al. 2015). *Sclerospora graminicola* is known to be a highly variable pathogen because of the existence of sexual stages in its lifecycle which facilitates pathogen to undergo rapid genetic recombination leading to the emergence of new pathotypes with a high degree of virulence

(Thakur et al. 2009). The emergence of more virulent pathotypes of *S. graminicola* in the recent past has resulted in the susceptibility of pearl millet cultivars hitherto resistant to existing pathotypes.

Thus, the usage of the resistant cultivars is the most powerful approach for better disease management. However, the source of sustainable and prolonged resistance is not available and new hybrids need to be developed continuously. In such hybrid breeding, the foremost requirement is resistance to DM pathogen but limited information is available about the mechanism of resistance to downy mildew disease in pearl millet. At implementing a hybrid breeding program resistance to disease should be one of the key selection criteria and potential parents should be tested for disease reactions to DM pathogens at identified disease hotspots globally (Kanfany et al. 2018). Likewise, 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. Recently, it has witnessed massive advancements in the stream of plant genomics and its deployment with conventional breeding in the form of the development of HHB 67 improved hybrid variety (Hash et al. 2006).

Among the molecular markers that have been discovered in the recent past are microsatellites (SSRs), single nucleotide polymorphisms (SNPs) and microarray derived markers like single feature polymorphisms (SFPs), diversity array technology (DArT) markers and NGS-based advanced markers (Supriya et al. 2011; Varshney et al. 2014; Singh et al. 2015, 2018). SSRs have been considered as the markers of choice because of their inherited genetic attributes of high abundance within the eukaryotic and prokaryotic genomes, reproducibility, transferability, amenable to high throughput automation and still preferred because of their potential in tracking alleles in molecular breeding programs (Singh et al. 2011, 2012; Kumar et al. 2018). Expressed sequence tags based microsatellite (EST-SSR) markers have been developed from publically available EST database (dbEST) in a cost-efficient manner and used for genetic evaluation in several plant species (Singh et al. 2019). For pearl millet, several hundreds of SSR markers have been developed (Senthilvel et al. 2008; Rajaram et al. 2013) and many consensus linkage maps have been constructed (Kumar et al. 2016; Anuradha et al. 2017) as a consequence of consistent efforts by many of the research scientists.

Potential RFLP molecular markers have been employed in detection quantitative trait loci (QTLs) associated with genomic regions controlling downy mildew resistance (DMR) in pearl millet (Jones et al. 1995). There many other success stories of QTL mapping for several virulent isolates of *S. graminicola* from Indian and African origin (Azhaguvel 2001; Nepolean 2002; Gulia 2004). Recently, EST-SSR DNA markers were employed to dissect QTLs for DMR in pearl millet by Taunk et al. (2018). Since the pathogen-host is a crop plant of marginal environments of arid and semi-arid regions, the use of resistant cultivars is the suitable, preferably useful, eco-friendly and cost-effective ways for the management of downy mildew in pearl millet. Hence, breeding for higher productivity and resistance to foliar diseases has been an underlying concern of crop breeders. Although in the last decade elite cultivars resistant to downy mildew have been developed worldwide through conventional breeding practices, frequent emergence of new virulent pathotypes of *S. graminicola* poses serious challenges to the breeding community in phenotypic selection. With this background, the current study was undertaken to map downy mildew resistance QTLs to virulent strains of the downy mildew pathogen *S. graminicola*.

Materials and methods

Plant material

Recombinant-inbred lines (RILs) mapping population of 187 F₈ progenies derived from cross ICMB 89111-P6 × ICMB 90111-P6 was used to construct SSRs based linkage map and screened for downy mildew resistance at pearl millet breeding unit, dryland cereals, ICRISAT, Patancheru, India. ICMB 89111B-P6 is a downy mildew susceptible elite parent used as a D2 dwarf maintainer of the A1 cytoplasmic male sterility (CMS) system (Rai and Rao 1998). This is a moderately photoperiod-sensitive parental line characterized by high tillering ability and site-specific downy mildew resistance. It is also used as a maintainer line of recently released pearl millet hybrids HHB 94 and RHB 121.

ICMB 90111-P6 is a resistance donor parent, genetically tall, weak restorer of male-fertility for the A1 CMS system (Rai and Rao 1998). It is characterized

by high tillering capacity and highly stable downy mildew resistance. It is also moderately photoperiod-sensitive. ICMP 90111-P6 is the pollinator of hybrid MH 143 and a maintainer of the A_{egg} CMS system.

Greenhouse screening for downy mildew resistance

Three new isolates including Sg445 collected from Banaskantha location (Gujarat, India), Sg519 from Rewari (Haryana, India) and Sg526 isolate from Jodhpur (Rajasthan, India) were collected during 2009–2010 from the A_1 zone in India (Sharma et al. 2014). An effective greenhouse screening technique was used to identify resistance in RILs mapping population and in contrast parents to different pathotypes of *S. graminicola* (Singh et al. 1993). Phenotyping of mapping population of 187 RIL progenies and their parent genotypes along with susceptible check (7042(S)) was performed during *Kharif* season 2013. Seeds of susceptible control entry 7042(S), and 187 F_8 RILs segregating for downy mildew resistance from the cross ICMB 89111-P6 (susceptible) \times ICMB 90111-P6 (resistant) were planted in plastic pots (12 seeds/pot) containing sterilized soil-sand-farmyard manure (FYM) potting mixture (2:1:1 by volume) in a randomized block design and maintained in the greenhouse at 35 °C till seedling stage.

Artificial disease epiphytotics were created by spraying the inoculum having a sporangial concentration of $1 \times 10^6 \text{ ml}^{-1}$ over the seedlings at coleoptile to the first-leaf stage by air-driven, nebulizer till run-off ensuring that each plantlet has received homogeneous pathogen load. The sprayed plantlets were covered with polyethylene bags instantly to provide a highly humid atmosphere required for infection and incubated in the dark at 20 °C for 18–22 h. The inoculated plants were maintained at 25 ± 2 °C with regular misting to create high humidity (>90% RH) and leaf wetness for disease development for 14 days. Downy mildew incidences was recorded at 16 days after shifting to the mist chamber. Downy mildew incidence (DMI) or disease severity was calculated using formula devised by James (1983), as the number of diseased plants expressed like; $\text{DMI} = (\text{number of diseased plants}/\text{total number of plants}) \times 100$. Disease reaction was scored as: highly susceptible $\text{DMI} > 80\%$, susceptible 50 to 80% DMI, moderately susceptible 25 to 50% DMI, moderately resistant > 10 to 25% DMI and resistant $\leq 10\%$ DMI (James 1983).

Genomic DNA extraction and SSR genotyping

Genomic DNA was extracted from newly emerged leaves of the parent genotypes and RIL population (F_8 generation) using the modified cetyl-trimethylammonium bromide (CTAB) protocol (Mace et al. 2003). Quality of extracted DNA was checked on 0.8% agarose gels and concentration was accurately quantified by using Nanodrop spectrophotometer (Nanodrop 8000). The working DNA samples with 5 ng/ μl concentrations were adjusted using double distilled water for genotyping experiment. The expressed sequence tags (ESTs) derived simple sequence repeat (SSR) markers (Senthilvel et al. 2008; Yadav et al. 2007; Rajaram et al. 2013) and genomic SSRs (Qi et al. 2004; Budak et al. 2003) developed under previous research programs were used to screen for polymorphism using contrast parent genotypes. The polymorphic markers were identified, selected and eventually used in genotyping of the RIL population.

SSR markers were amplified in a 10 μl PCR reaction volume consisting 10–15 ng of template DNA, 2 pmol of each (forward and reverse) primer, 2 mM MgCl_2 , 0.4mM of each dNTP, 1 \times reaction buffer, and 0.2 U Taq polymerase (Bioline). PCR parameters were set as: denaturation at 94 °C for 5 min, followed by 10 subsequent cycles of denaturation at 94 °C for 15 s, annealing at 61 °C to 51 °C (touch-down cycles) for 30 s, and extension at 72 °C for 30 s, followed by 40 cycles of denaturation at 94 °C for 10s, annealing at 54 °C for 30s, and extension at 72 °C for 30 s, followed by final extension at 72 °C for 20 min. PCR amplification was checked on 1.5% agarose gel and PCR products were resolved by capillary electrophoresis on an ABI3730xl sequencer and their sizes were determined using GeneMapperv4.0 software (Applied Biosystems, USA).

Linkage map construction

The genotyping scores for all microsatellite markers were transformed into genotype codes in accordance with the scores of the parent genotypes. Genotyping data for 53 polymorphic g/EST SSR markers (Rajaram et al. 2013) were used to construct genetic linkage map and linkage groups were obtained using JoinMap v4.1 (Stam 1993) at logarithm of odds (LOD) threshold value of > 3.0 and recombination fraction of 0.5. The order of markers in every linkage group (LG) was decided through RECORD software with Haldane mapping function. The recombination

fraction was converted into map distances in centimorgans (cM) using the Haldane mapping function (Haldane 1919). The inter-marker distances calculated from Mapmaker were used to construct a linkage map using Map Chart 2.1 (Voorrips 2002).

Quantitative trait loci (QTLs) analysis

For QTL mapping, the linkage map constructed with SSRs marker data from 187 F_8 RILs derived from the cross ICMB 89111-P6 \times ICMB 90111-P6 was used. The entry means of raw data recorded for disease reaction percentage were used for QTL analysis, which was accomplished through composite interval mapping (CIM) approach (Zeng 1994) using a LOD of 3.0 as the threshold value for QTL significance using WinQTL Cartographer, version 2.5 (Wang et al. 2007). CIM was performed using Model 6, scanning intervals of 2.0 cM between markers and putative QTL with a window size of 10.0 cM. The number of marker cofactors for the background control was set by forward-backward stepwise regression. Automatically “Locate QTLs” option was used with a minimum of 5 cM between QTL to define a QTL region and, if the peak’s distance was less than 5.0 cM, then the highest peak was considered to locate QTL. Permutations for 1000 times were also performed while determining the QTL using WinQTL Cartographer V 2.5 using the option “permutations times” with 0.05 significance level.

Results

Downy mildew phenotyping of RILs mapping population

Male parental line ICMB 90111-P6 was highly resistant and exhibited very low downy mildew incidence (DMI) against the two pathotype-isolates Sg519 (1.67%) and Sg526 (7.66%) and moderately resistant to isolate Sg445 (10.73%). Female parental line ICMB 89111-P6 was highly susceptible to all the three pathotype-isolates Sg445 (97.54%), Sg519 (97.65%), and Sg526 (95.33%). The individual comparisons of average downy mildew disease incidence on the 187 RILs made among three pathotype-isolates. The pathotype-isolate Sg445 of

S. graminicola collected from Banaskantha caused the greatest disease incidence (59.54%) followed by isolate Sg519 from Rewari (40.55%) (Fig. 1). The pathotype-isolate Sg526 collected from Jodhpur location with mean DMI 37.47% was observed to be the least virulent.

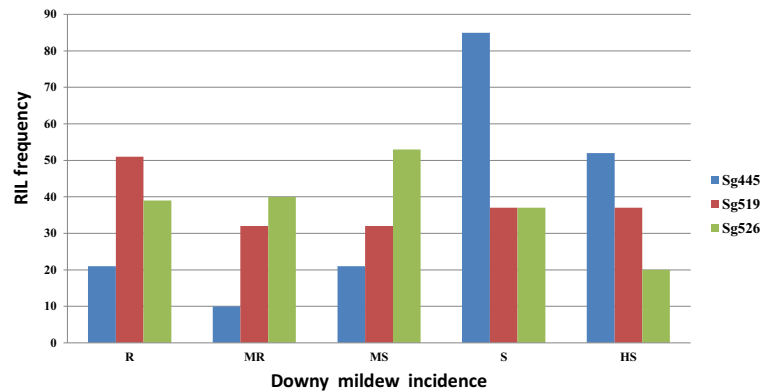
SSR marker analysis in the mapping population

A total of 468 (121 genomic and 347 expressed sequence tags) SSR markers were screened across the contrast (resistant and susceptible) parent genotypes ICMB 89111-P6 (P1 susceptible) and ICMB 90111-P6 (P2 resistant) for the genetic polymorphism. Out of 468 markers, only 88 (18.8%) were found to show robust polymorphism in the polymorphism survey. These 88 (39 genomic SSRs and 49 EST SSRs) polymorphic SSR markers were used for genotyping experiment with 187 F_8 recombinant inbred lines (RILs) mapping population (Fig. 2a, b).

Development of a genetic map

Genotyping data obtained for all the 88 markers from RILs was subjected to Chi-square (χ^2) test to calculate the segregation ratio. Out of 88 markers, only 53 markers revealed significant χ^2 values when compared with table value of 6.64 at the 1.0% probability level. The remaining 35 markers (39.7%) deviated significantly from the expected ratio at 1.0% probability level. The significant χ^2 values were ranged from 6.65 (Xipes0203) to 42.2 (Xipes0011). However, these markers were used to construct a genetic map. Linkage map analysis of the 88 loci resulted in the mapping of 53 marker loci on 7 linkage groups (LGs) with total map length of 903.8 cM (Fig. 3a). The map length of individual linkage groups ranged from a minimum of 23.0 cM (LG3) to a maximum of 241.1 cM (LG1). The average intermarker distance was 18.1 cM, with an average density of 0.05 markers/cM. The total number of mapped loci per linkage group (LG) ranged from 4 (LG3) to 14 LG1. Constructed genetic map was compared with the reference consensus maps earlier developed and a good congruence was found between the maps for common markers and their order with few exceptions (Rajaram et al. 2013). The names of the linkage groups (LGs) of the developed genetic map were

Fig. 1 Frequency distribution of downy mildew incidence (%) among F_8 RIL progenies derived from (ICMB 89111B-P6 \times ICMB 90111B-P6) cross. R = *resistant*; MR = *moderately resistant*; MS = *moderately susceptible*; S = *susceptible*; HS = *highly susceptible*



assigned according to the LGs of the reference map developed by Rajaram et al. (2013).

Identification of QTL for downy mildew resistance

For identification of QTL controlling downy mildew resistance, QTL analysis was done in RIL mapping population using the corresponding genetic mapping data and precision phenotyping data for three isolates of downy mildew pathogen obtained by converting the primary data of downy mildew incidence percentage into resistance percentage. The QTL analysis was performed by composite interval mapping with windows QTL cartographer v2.5 using a LOD score of 3.0 as the threshold value at 0.05 significance levels and walk speed of 1 cM with 1000 permutations for QTL significance. As a result, a total of five QTLs governing resistance to three downy mildew pathotype-isolates were identified on one linkage group, LG4 (Fig. 3b). A major QTL was detected associated with resistance to Sg445 isolate with a LOD value of 5.11 on the LG4 explaining 16.7% of the observed phenotypic variation for downy mildew reaction in the RIL progenies (Table 1). Two major QTLs were found associated with resistance to Sg519 pathotype-isolate with a LOD value of 10.14 and 16.03 were also mapped on the LG4 (Fig. 3b) explaining 76.95% and 78.0% of the observed phenotypic variation, respectively, for downy mildew reaction. Two major QTLs associated with resistance to Sg526 pathotype-isolate with a LOD value 10.48 and 9.80 were mapped on the LG4 explaining 70.71% and 47.7% of the observed phenotypic variation, respectively (Table 1). The position of the QTLs detected for three different pathotype-isolates on LG 4, namely Sg 445, Sg 519 and Sg 526 with their LOD and additive effects are represented in Fig. 4.

Discussion

In the present investigation, simple sequence repeat (SSR) marker system enabled identification of marker loci linked to downy mildew resistance in the (ICMB 89111-P6 \times ICMB 90111-P6)-derived RIL mapping population in a cost-effective manner. The EST-SSR markers used in this study were exploited from the earlier (Senthilvel et al. 2008; Yadav et al. 2007; Rajaram et al. 2013) reported pearl millet genetic resources without any expenditure. Such a publically available EST (dbEST) database for plant species offers an excellent opportunity to cost-efficient development of the informative genic microsatellite (EST-SSR) markers for various genetic studies in cereal grasses (Singh et al. 2014, 2019).

Initially, RFLP molecular markers were used for the construction of the linkage maps as genetic resources for molecular breeding in pearl millet (Liu et al. 1994; Hash et al. 1995). Time-to-time more, markers were added into the previously developed linkage maps using other mapping populations of pearl millet (Gulia 2004). Molecular linkage map of pearl millet integrating DARt and SSR markers was constructed under subsequent molecular breeding programs (Supriya et al. 2011). A set of 174 EST-based SSR markers were employed to develop a consensus linkage map using recombinant inbred lines (RILs) mapping population at the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Hyderabad, India (Rajaram et al. 2013). Genotyping-by-sequencing (GBS) has also been used to construct the pearl millet genetic map (Moumouni et al. (2015).

The availability of densely-saturated genetic molecular maps those are feasible by microsatellites, DARt, SNPs and other marker system pipelines will offer breeders and geneticists with an enviable tool to identifying different genomic regions of interest, which in turn will enhance the

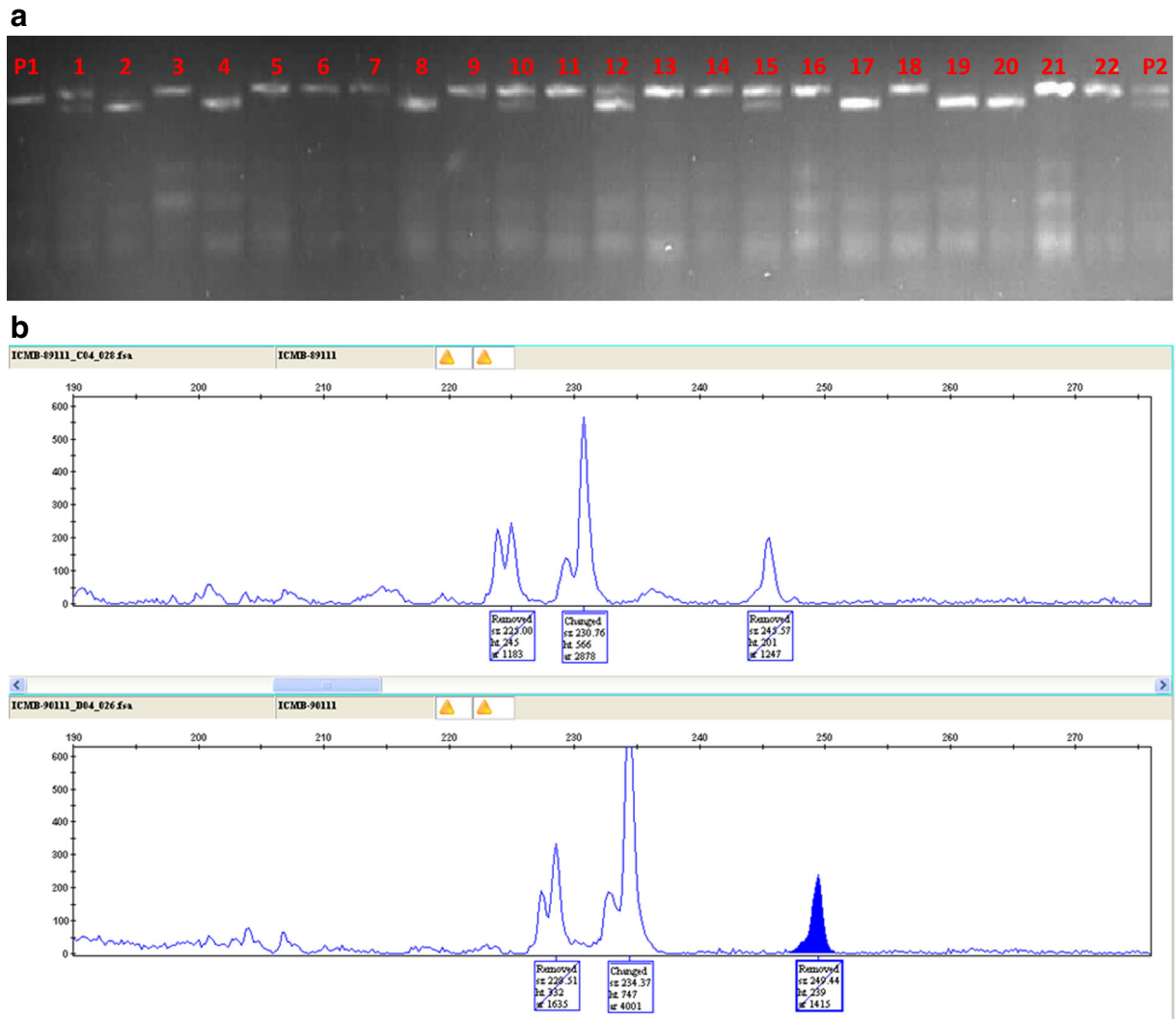


Fig. 2 a) Agarose gel image showing length polymorphism among the mapping population parental lines ICMB 89111B-P6 (P1) and ICMB 90111-P6 (P2) and select mapping population progenies (line 1–22) using Xpsmp2237 marker. **b)** GeneMapper

profile depicting polymorphism among mapping population parent lines ICMB 89111B-P6 (P1) and ICMB 90111-P6 (P2) with Xipes0208 marker

efficiency of marker-assisted breeding (Ambawat et al. 2016). QTL mapping is a prerequisite for the identification of molecular markers associated with tolerance/resistance to abiotic/biotic stresses and agronomically important traits (Kumar et al. 2018). Subsequently several QTLs were reported for different agronomic traits of interest using various markers such as; for mineral (Fe and Zn) nutrients (Kumar et al. 2016, 2018), grain and stover yield under terminal drought stress conditions (Yadav et al. 2002), water-use traits (Apama et al. 2015), grain yield under variable post-flowering moisture conditions (Bidinger et al. 2007), terminal drought tolerance (Sharma et al.

2011), domestication traits (Poncet et al. 2002), rust and pyricularia leaf spot disease resistance (Morgan et al. 1998), and downy mildew resistance (Hash et al. 1995; Jones et al. 1995, 2002; Gulia 2004; Taunk et al. 2018).

The frequent emergence of new pathogen stains due to genetic recombination during sexual stages in life cycle of *S. graminicola* makes it important to identify and map new sources of resistance for its use in pearl millet breeding programs (Sharma et al. 2014). A reliable phenotypic data is vital for the identification of QTLs/genes which will be potentially useful in marker-assisted selection (MAS). Proper

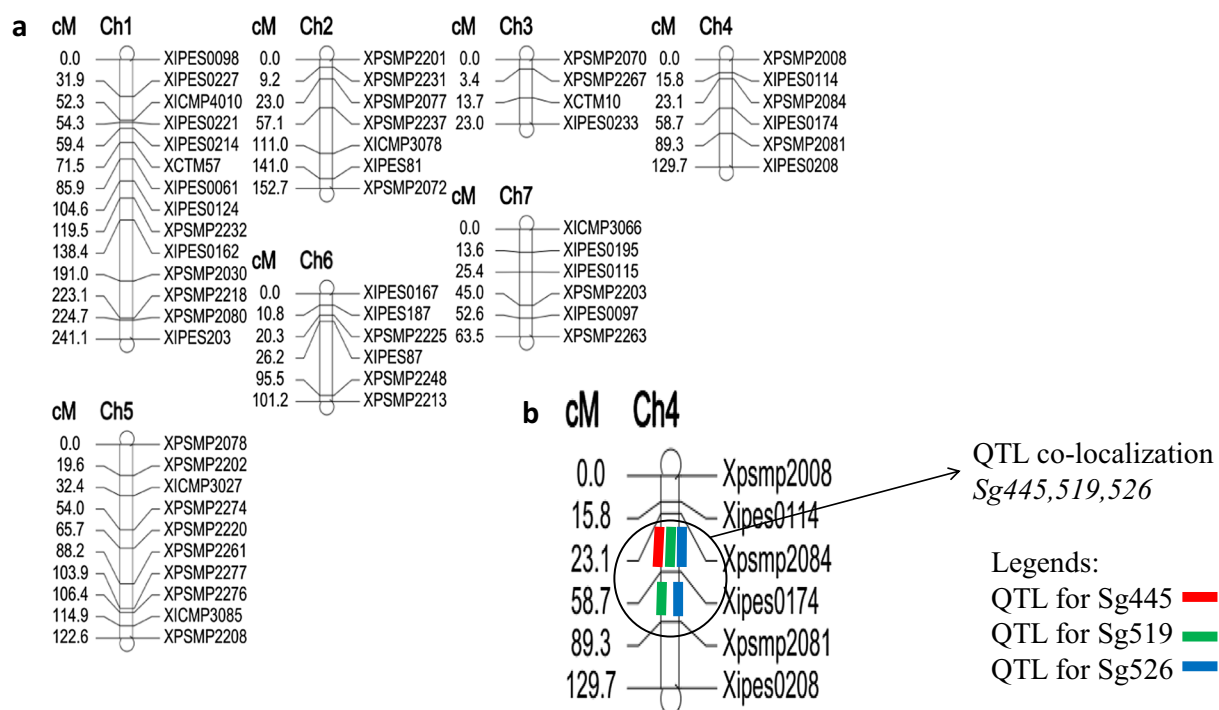


Fig. 3 Representation of **a**) linkage map; and **b**) QTL positions for resistance to three pathotype-isolates of the downy mildew pathogen *Sclerospora graminicola* based on (ICMB 89111-P6 ×

ICMB 90111-P6) RIL mapping population. Note: The marker name and genetic distance (cM) are listed on right and left sides, respectively

randomization, suitable number of replications and controlling other environmental variation are important factors for precise and accurate estimates of phenotypic values for QTL mapping (Paterson et al. 1991). In order to achieve this, in the present study precision phenotyping protocol (Singh et al. 1993) was used, with three replications of RIL population and checks using time isolation in order to reduce environmental effects.

In this current study 53 out of 88 co-dominant marker loci revealed non-significant χ^2 values when compared with table value of 6.64 at the 1% probability level. The remaining 35 markers (39.7%) deviated significantly

from the expected ratio at 1% probability level. As RIL populations have the highest potential for such distortions due to repeated generations of selection forces (Singh et al. 2007), which can be accentuated by the loss of vigor with enforced inbreeding. It has been suggested that such segregation distortion is likely to be high in pearl millet because of its protogynous nature and sensitivity to inbreeding depression (Kumar et al. 2016). In linkage map analysis, the position and order of the markers were found to be congruence with the earlier developed consensus maps for pearl millet except for swapping of some marker orders within some blocks

Table 1 QTLs for downy mildew resistance trait detected by composite interval mapping in (ICMB 89111B-P6 × ICMB 90111B-P6)-RIL mapping population

Pathotype-isolate	Likage group	QTLs detected	Flanking Marker	Position (cM)	LOD score	Additive effect	R ²	Inheritance
Sg445	LG4	1	Xpsmp2084-Xipes0174	53.1	5.11	-10.94	0.167	From ICMB 90111-P6
Sg519	LG4	1	Xpsmp2084-Xipes0174	43.1	16.03	-28.11	0.780	From ICMB 90111-P6
		2	Xipes0174-Xpsmp2081	70.7	10.14	-27.68	0.769	From ICMB 90111-P6
Sg526	LG4	1	Xpsmp2084-Xipes0174	45.1	10.48	-20.89	0.707	From ICMB 90111-P6
		2	Xipes0174-Xpsmp2081	66.7	9.80	-15.91	0.477	From ICMB 90111-P6

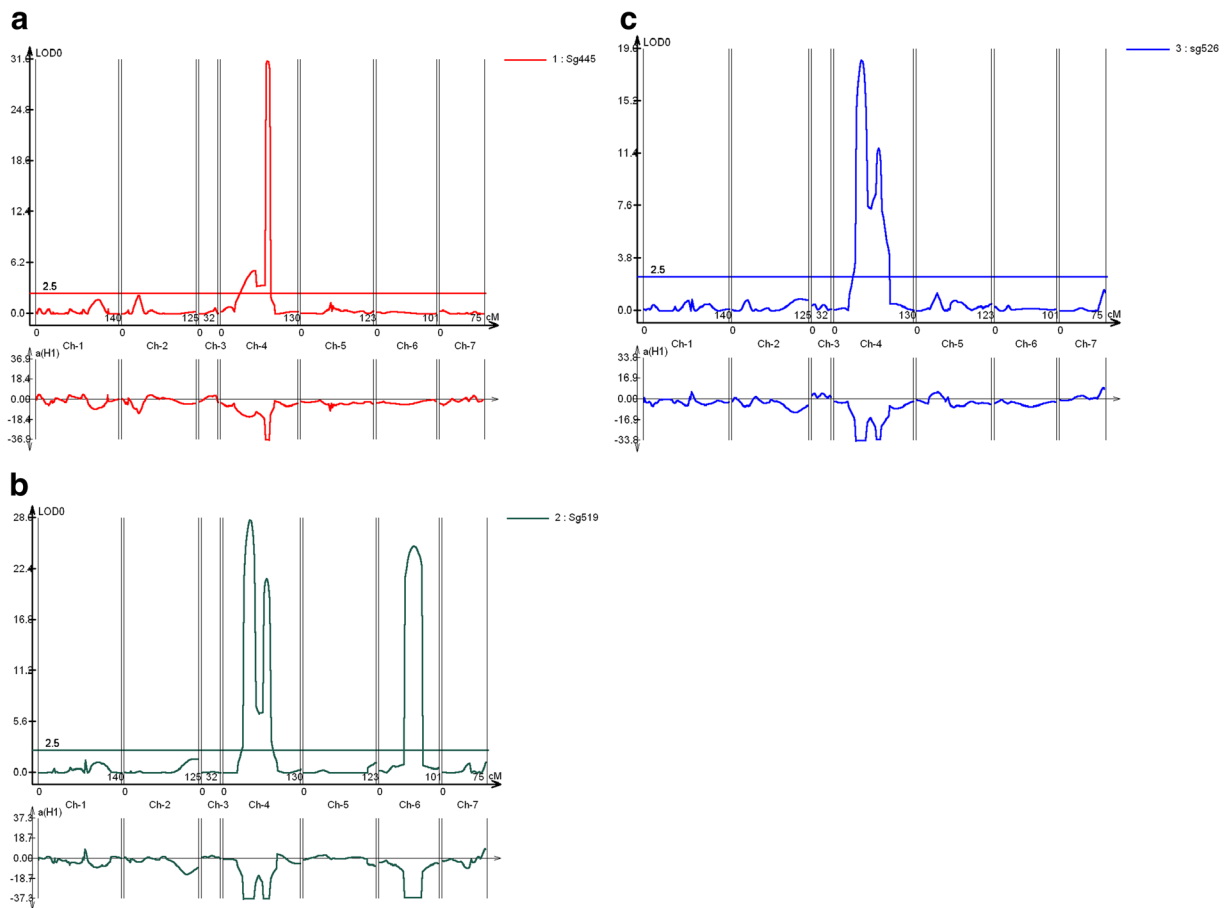


Fig. 4 a) QTL for Sg445 in red color showing LOD score and additive effect on linkage group 4; b) QTLs for Sg519 in green color showing LOD values and additive effects on linkage group

4; and c) QTLs of Sg526 in blue color showing LOD and additive effects on linkage group 4

on a few linkage groups (Yadav et al. 2007; Rajaram et al. 2013). Such discordance in terms of marker order among diverse genetic maps is not unexpected, as genetic mapping reflects merely a gesture of the relative positions and genetic distances of markers to each other (Sourdille et al. 2004; Ambawat et al. 2016). Under the present investigation, segregation distortion was observed for 39.7% of the total marker loci assessed, which is similar to earlier research reports (Supriya et al. 2011).

The downy mildew resistance QTLs for all the three pathotype-isolates were detected on LG4. This is in accordant with the previous studies, and also suggests that no single QTL is effective against a range of pathogen strains (Gulia 2004; Taunk et al. 2018). Strain-specific resistance appears to be a prime mechanism for downy mildew resistance in pearl millet (Taunk et al. 2018). These QTLs need to be stacked together to impart resistance against multiple

pathotype-isolates originating from different geographical locations. In another study, the QTLs for downy mildew resistance were found to be on LG1 and LG4, at different positions against different isolates of *S. graminicola* (Taunk et al. 2018). This reveals considerable differences within the genomic constitution of different pathogen strains of diversified origin. These facts have been endorsed by past studies wherein the differences between pathotype-isolates collected from India and Africa were observed (Azhaguvel 2001). It has been articulated that entire grass (pearl millet, sorghum, rice, wheat, foxtail millet, and others) family shows a substantial level of genomic synteny and collinearity viz.; gene number and gene order are evolutionarily conserved (Varshney et al. 2002; Singh et al. 2011). Thus, the QTL identified in this study may provide a potential tool to identify various genomic regions exploited in other crops as well (Ambawat

et al. 2016; Taunk et al. 2018). The major effect QTLs reported from this study may govern resistance against specific pathotype-isolate of downy mildew pathogen such as those from Rajasthan, Haryana and Gujarat states of India. The host-pathogen interaction may be specific to downy mildew pathotype- isolates from these regions. However, these are considerably large pearl millet growing area, together accounting for more than 70% of the total pearl millet cropped area in India. The co-localized QTLs offer opportunity for simultaneous improvement of downy mildew resistance levels for all the three pathotype-isolates. Deployment of these QTLs may offer an effective host-plant resistance (HPR) strategy for downy mildew management.

Conclusion

The present study reports three large effect downy mildew resistance associated QTLs using a biparental RIL mapping population segregating for disease resistance against three virulent isolates/strains of the pathogen *S. graminicola*. The inheritance of these QTLs showed that the resistant parent ICMB 90111-P6 contributed the resistance alleles. The observed phenotypic variance ranged from 16.7 to 78.0% for the five QTLs. These QTL for downy mildew resistance can directly facilitate understanding of the mechanisms for disease resistance. After necessary validation, the reported major effect QTLs may be used to impart resistance against the major pathotype-isolates of downy mildew pathogen from India using various marker-assisted selection (MAS) strategies.

Acknowledgments The authors acknowledge the help provided by pearl millet breeding and pathology staff at ICRISAT-Patancheru. This work has been published as part of the CGIAR Research Program on Dryland Cereals.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest directly or indirectly and informed consent to publish this study and that the manuscript complies with the ethical standards of the journal.

Research involving human participants and/or animals Not applicable to this study, did not work with humans or animals.

Informed consent Not applicable to this study, did not work with humans.

References

- Ambawat, S., Senthilvel, S., Hash, C. T., Nepolean, T., Rajaram, V., Eshwar, K., Sharma, R., Thakur, R. P., Rao, V. P., Yadav, R. C., & Srivastava, R. K. (2016). QTL mapping of pearl millet rust resistance using an integrated DArT-and SSR-based linkage map. *Euphytica*, *209*, 461–476.
- Anuradha, N., Satyavathi, C. T., Bharadwaj, C., Nepolean, T., Sankar, S. M., Singh, S. P., Meena, M. C., Singhal, T., & Srivastava, R. K. (2017). Deciphering genomic regions for high grain iron and zinc content using association mapping in pearl millet. *Frontiers in Plant Science*, *8*, 412.
- Apama, K., Nepolean, T., Srivastava, R. K., Kholová, J., Rajaram, V., Kumar, S., Rekha, B., Senthilvel, S., Hash, C. T., & Vadez, V. (2015). Quantitative trait loci associated with constitutive traits controlling water use in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Plant Biology*, *17*(5), 1073–1084.
- Azhaguvel P. (2001). Linkage map construction and identification of QTLs for downy mildew (*Sclerospora graminicola*) resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.], Ph.D. Thesis, TNAU, Coimbatore.
- Bidinger, F. R., Nepolean, T., Hash, C. T., Yadav, R. S., & Howarth, C. J. (2007). Quantitative trait loci for grain yield in pearl millet under variable postflowering moisture conditions. *Crop Science*, *47*(3), 969–980.
- Brunken, J. N. (1977). A systematic study of *Pennisetum* sect. *Pennisetum* (Gramineae). *American Journal of Botany*, *64*, 161–176.
- Budak, H., Pedraza, F., Cregan, P. B., Baenziger, P. S., & Dweikat, I. (2003). Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Science*, *43*, 2284–2290.
- DES, (2012–13). Directorate of Economics and Statistics, Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India.
- FAOSTAT, (2017) FAO Database. Available at <http://faostat.fao.org>. Accessed 10 June 2018
- Gulia, S. K. (2004). QTL mapping for improvement of downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance (DMR) in pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrid parental line ICMB 89111. Ph.D. Thesis, CCS HAU, Hisar, India.
- Haldane, J. (1919). The combination of linkage values and the calculation of distance between the loci of linked factors. *Journal of Genetics*, *8*, 299–309.
- Hash, C. T., Cavan, G. P., Bidinger, F. R., Howarth, C. J., & Singh, S. D. (1995). Downy mildew resistance QTLs from a seedling heat tolerance mapping population. *International Sorghum and Millets Newsletter*, *36*, 66–67. ISSN 0584-1321.
- Hash, C. T., Sharma, A., Kolesnikova-Allen, M. A., Singh, S. D., Thakur, R. P., Raj, A. B., Rao, M. R., Nijhawan, D. C., Beniwal, C. R., Sagar, P., & Yadav, H. P. (2006). Teamwork delivers biotechnology products to Indian small-holder crop-livestock producers: Pearl millet hybrid “HHB 67 improved” enters seed delivery pipeline. *Journal of SAT Agricultural Research*, *2*, 1–3.
- James, W. C. (1983). Crop loss assessment. In: Plant Pathologist. Pook book 2nd Edn. (Johnson, A. and C. Boths, Eds.). Common wealth Mycological Institute, Kew, pp: 130–140.

- Jones, E. S., Liu, C. J., Gale, M. D., Hash, C. T., & Witcombe, J. R. (1995). Mapping quantitative trait loci for downy mildew resistance in pearl millet. *Theoretical and Applied Genetics*, *91*, 448–456.
- Jones, E. S., Breese, W. A., Liu, C. J., Singh, S. D., Shaw, D. S., & Witcombe, J. R. (2002). Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Filled and greenhouse screens detect the same QTL. *Crop Science*, *42*, 1316–1323.
- Kanfany, G., Zoclanclounon, Y. A. B., Tongoona, P., Danquah, A., Offei, S. K., Fofana, A., Thiaw, C., Danquah, E. Y. & Cisse, N., (2018). Evidence of pathogenic variation in *Sclerospora graminicola* populations from pearl millet growing regions in Senegal. *Journal of Plant Pathology*, *100*(3), 429–434.
- Kumar, S., Hash, C. T., Thirunavukkarasu, N., Singh, G., Rajaram, V., Rathore, A., Senapathy, S., Mahendrakar, M. D., Yadav, R. S., & Srivastava, R. K. (2016). Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Frontiers in Plant Science*, *7*, 1636.
- Kumar, S., Hash, C. T., Nepolean, T., Satyavathi, C. T., Singh, G., Mahendrakar, M. D., Yadav, R. S., & Srivastava, R. K. (2017). Mapping QTLs controlling flowering time and important agronomic traits in pearl millet. *Frontiers in Plant Science*, *8*, 1731.
- Kumar, S., Hash, C. T., Nepolean, T., Mahendrakar, M. D., Satyavathi, C. T., Singh, G., Rathore, A., Yadav, R. S., Gupta, R., & Srivastava, R. K. (2018). Mapping grain iron and zinc content quantitative trait loci in an Inia-di-derived immortal population of pearl millet. *Genes*, *9*, 248.
- Liu, C. J., Witcombe, J. R., Pittaway, T. S., Nash, M., Hash, C. T., Busso, C. G., & Gale, M. D. (1994). A RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). *Theoretical and Applied Genetics*, *89*, 481–487.
- Mace, E. S., Buhariwalla, K. K., Buhariwalla, H. K., & Crouch, J. H. (2003). A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter*, *21*, 459–460.
- Manning, K., Pelling, R., Higham, T., Schwenniger, J. L., & Fuller, D. Q. (2011). 4500-year old domesticated pearl millet (*Pennisetum glaucum*) from the Tilemsi Valley, Mali: New insights into an alternative cereal domestication pathway. *Journal of Archaeological Science*, *38*, 312–322.
- Morgan, R. N., Wilson, J. P., Hanna, W. W., & Ozias-Akins, P. (1998). Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet. *Theoretical and Applied Genetics*, *96*(3–4), 413–420.
- Moumouni, K. H., Kountche, B. A., Jean, M., Hash, C. T., Vigouroux, Y., Haussmann, B. I., & Belzile, F. (2015). Construction of a genetic map for pearl millet, *Pennisetum glaucum* (L.) R. Br., using a genotyping-by-sequencing (GBS) approach. *Molecular Breeding*, *35*, 5.
- Nepolean, T. (2002). Identification of QTLs for yield and its component traits and downy mildew [*Sclerospora graminicola* (Sacc.) J. Schrot.] resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Ph.D. thesis, TNAU, Coimbatore, India.
- Paterson, A. H., Damon, S., Hewitt, J. D., Zamir, D., Rabinowitch, H. D., Lincoln, S. E., Lander, E. S., & Tanksley, S. D. (1991). Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. *Genetics*, *127*, 181–197.
- Poncet, V., Martel, E., Allouis, S., Devos, K., Lamy, F., Sarr, A., & Robert, T. (2002). Comparative analysis of QTLs affecting domestication traits between two domesticated \times wild pearl millet (*Pennisetum glaucum* L., Poaceae) crosses. *Theoretical and Applied Genetics*, *104*(6–7), 965–975.
- Qi, X., Pittaway, T. S., Lindup, S., Liu, H., Waterman, E., Padi, F. K., Hash, C. T., Zhu, J., Gale, M. D., & Devos, K. M. (2004). An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. *Theoretical and Applied Genetics*, *109*, 1485–1493.
- Rai, K. N., & Rao, A. S. (1998). Registration of pearl millet cytoplasmic-nuclear male-sterile line ICMA-5. *Crop Science*, *38*, 556.
- Rajaram, V., Nepolean, T., Senthilvel, S., Varshney, R. K., Vadez, V., Srivastava, R. K., Shah, T. M., Supriya, A., Kumar, S., Kumari, B. R., & Bhanuprakash, A. (2013). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs. *BMC Genomics*, *14*, 159.
- Sanou, J., Bayala, J., Bazie, P., & Teklehaimanot, Z. (2012). Photosynthesis and biomass production by millet (*Pennisetum glaucum*) and taro (*Colocasia esculenta*) grown under baobab (*Adansonia digitata*) and néré (*Parkia biglobosa*) in an agroforestry parkland system of Burkina Faso (West Africa). *Experimental Agriculture*, *48*, 283–300.
- Senthilvel, S., Jayashree, B., Mahalakshmi, V., Kumar, P. S., Nakka, S., Nepolean, T., & Hash, C. T. (2008). Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. *BMC Plant Biology*, *8*, 119.
- Sharma, P. C., Sehgal, D., Singh, D., Singh, G., & Yadav, R. S. (2011). A major terminal drought tolerance QTL of pearl millet is also associated with reduced salt uptake and enhanced growth under salt stress. *Molecular Breeding*, *27*, 207–222.
- Sharma, R., Gupta, S. K., Kadvani, D. L., Shivpuri, A., & Rai, K. N. (2014). New virulent pathotypes of *Sclerospora graminicola* and resistance sources in pearl millet for A1 zone in India. *The Indian Journal of Agricultural Sciences*, *84*(6), 707–710.
- Sharma, R., Upadhyaya, H. D., Sharma, S., Gate, V. L., & Raj, C. (2015). New sources of resistance to multiple pathotypes of *Sclerospora graminicola* in the pearl millet mini core germplasm collection. *Crop Science*, *55*, 1619–1628.
- Shivhare, R., & Lata, C. (2017). Exploration of genetic and genomic resources for abiotic and biotic stress tolerance in pearl millet. *Frontiers in Plant Science*, *7*, 2069.
- Singh, S. D., King, S. B., & Werder, J. (1993). Downy mildew disease of pearl millet. *Information Bulletin no. 37*, ICRISAT, Patancheru, India.
- Singh, K., Ghai, M., Garg, M., Chhuneja, P., Kaur, P., Schnurbusch, T., Keller, B., & Dhaliwal, H. S. (2007). An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum \times *T. monococcum* RIL population. *Theoretical and Applied Genetics*, *115*, 301–312.*
- Singh, R. K., Singh, R. B., Singh, S. P., & Sharma, M. L. (2011). Identification of sugarcane microsatellites associated to sugar content in sugarcane and transferability to other cereal genomes. *Euphytica*, *182*, 335–354.

- Singh, R. K., Singh, R. B., Singh, S. P., & Sharma, M. L. (2012). Genes tagging and molecular diversity of red rot susceptible/tolerant sugarcane hybrids using c-DNA and unigene derived markers. *World Journal of Microbiology & Biotechnology*, *8*, 1669–1679.
- Singh, R. B., Srivastva, S., Verma, A. K., Singh, B., & Singh, R. K. (2014). Importance and progress of microsatellite markers in sugarcane (*Saccharum* spp. hybrids). *Indian Journal of Sugarcane Technology*, *29*, 1–12.
- Singh, R. B., Singh, B., & Singh, R. K. (2015). Development of microsatellite (SSRs) markers and evaluation of genetic variability within sugarcane commercial varieties (*Saccharum* spp. hybrids). *International Journal of Advance Research*, *3*, 700–708.
- Singh, R. B., Singh, B., & Singh, R. K. (2018). Evaluation of genetic diversity in *Saccharum* species clones and commercial varieties employing molecular (SSRs) and physiological markers. *Indian Journal of Plant Genetic Resources*, *3*, 17–26.
- Singh, R. B., Singh, B., & Singh, R. K. (2019). Development of EST based simple sequence repeats (EST-SSRs) markers for *Saccharum* complex and allied cereal crops. *Industrial Crops and Products*, *128*, 38–47.
- Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G. L., Gay, G., Qi, L., Gill, B. S., Dufour, P., Murigneux, A., & Bernard, M. (2004). Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Functional & Integrative Genomics*, *4*, 12–25.
- Stam, P. (1993). Construction of integrated genetic linkage maps by means of computer package-JoinMap. *The Plant Journal*, *5*, 739–744.
- Supriya, A., Senthilvel, S., Nepolean, T., Eshwar, K., Rajaram, V., Shaw, R., Hash, C. T., Kilian, A., Yadav, R. C., & Narasu, M. L. (2011). Development of a molecular linkage map of pearl millet integrating DArT and SSR markers. *Theoretical and Applied Genetics*, *123*, 239–250.
- Taunk, J., Sehgal, D., Yadav, N. R., Howarth, C., Yadav, R. C., & Yadav, R. S. (2018). Mapping of easy to screen SSR markers for selection of RFLP markers-bracketed downy mildew resistance QTLs in pearl millet. *European Journal of Plant Pathology*, *151*, 401–411.
- Thakur, R. P., Rao, V. P., & Sharma, R. (2009). Temporal virulence change and identification of resistance in pearl millet germplasm to diverse pathotypes of *Sclerospora graminicola*. *Journal of Plant Pathology*, 629–636.
- Vadez, V., Hash, T., Bidinger, F. R., & Kholova, J. (2012). Phenotyping pearl millet for adaptation to drought. *Frontiers in Physiology*, *3*, 386.
- Varshney, R. K., Thiel, T., Stein, N., Langridge, P., & Graner, A. (2002). In silico analysis on frequency and distribution of microsatellites in ESTs of some cereal species. *Cellular and Molecular Biology Letters*, *7*(2A), 537–546.
- Varshney, R. K., Terauchi, R., & McCouch, S. R. (2014). Harvesting the promising fruits of genomics: Applying genome sequencing technologies to crop breeding. *PLoS Biology*, *12*(6), e1001883.
- Varshney, R. K., Shi, C., Thudi, M., Mariac, C., Wallace, J., Qi, P., et al. (2017). Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. *Nature Biotechnology*, *35*, 969–976.
- Voorrips, R. E. (2002). Map chart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*, 77–79.
- Wang, S., Basten, C. J., Zeng, Z. B. (2007). Windows QTL cartographer 2.5 <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>. Accessed 10 June 2018
- Yadav, R. S., Hash, C. T., Bidinger, F. R., Cavan, G. P., & Howarth, C. J. (2002). Quantitative trait loci associated with traits determining grain and Stover yield in pearl millet under terminal drought stress conditions. *Theoretical and Applied Genetics*, *104*, 67–83.
- Yadav, O. P., Mitchell, S. E., Zamora, A., Fulton, T. M., & Kresovich, S. (2007). Development of new simple sequence repeat markers for pearl millet. *Journal of SAT Agricultural Research*, *3*, 34.
- Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics*, *136*, 1457–1468.