

Original Research Article

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Screening of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] Genotypes against Blast Disease on the Basis of Disease Indexing and Gene-specific SSR Markers

Rahul Verma¹, M. K. Tripathi^{1*}, Sushma Tiwari¹, Niraj Tripathi³,
R. K. Pandya² and Prerana Parihar²

¹Department of Plant Molecular Biology & Biotechnology, ²Department of Plant Pathology, College of Agriculture, RVS Agricultural University, Gwalior, 474002 M.P. India

³Directorate of Research Services, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur-482004, India

*Corresponding author

ABSTRACT

The growth and productivity of pearl millet are harmfully influenced by an array of biotic and abiotic stresses. Blast is one of them as that cause enormous loss to the yield of pearl millet in India. The present study has been conducted with an objective to identify blast resistant genotypes on the basis of field as well as molecular data generated from gene specific SSR molecular markers. In the current research, a total number of 48 pearl millet genotypes have been screened against blast. In field screening, out of 48 genotypes 5 viz., P343, IP205, THAK1827, THAK0201 and THAK0285 have been found highly resistance, 5 were resistance, 20 moderate resistance, 8 susceptible and 10 highly susceptible. In molecular analysis, 4 germplasm lines viz., IP347, IP383, IP324 and IP351 including varieties, namely: THAK827, THAK0285 and THAK0201 have been found to be resistant against blast. Among the polymorphic SSR markers, the gene diversity varied from 0.5304 to 0.6302 for SSR markers FMBLESTSSR5 and FMBLESTSSR1 respectively with an average of 0.5804 and Polymorphic Information Content (PIC) values ranged between 0.4527 to 0.5620, for the markers FMBLESTSSR5 and FMBLESTSSR1 correspondingly with an average of 0.4975. The major allele frequency varied in range of 0.4792 for primer FMBLESTSSR1 to 0.6042 for primer FMBLESTSSR5 with a mean value of 0.5042. The resistant genotypes identified in this experiment may be utilized as donor of resistance gene against blast to develop improved genotypes which would situate as fence against spread of the disease to newer areas and thus it can boost production and productivity of pearl millet.

Keywords

Pearl millet, Blast, Genetic diversity, Polymorphism, Resistant, Susceptible

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an extremely cross-pollinated diploid ($2n = 2x = 14$) with vast genome size (~ 2352 Mbp) monocot belonging to the family Poaceae. It is believed to be originated from Dhar Tichitt,

a Saharan site in Mauritania of West Africa around 3500 B.C. (Amblard and Pernès, 1989). India is the greatest creator of pearl millet on the planet having a zone of 7.4 million ha and making of 9.13 million tons with productivity of 1237kg ha^{-1} (Directorate of Millets Development, 2019). Its grains are

high in supplement structure and are viewed as an inexpensive wellspring of energy when contrasted with staple cereals, for example, wheat, rice and maize regarding micronutrients *viz.*, Zinc and Iron (Kumar *et al.*, 2016) protein substance and amino corrosive arrangement for the asset helpless ranchers (Kanatti *et al.*, 2014). Truth be told, pearl millet is unrivalled in protein content, quality, protein energy proportion and energy levels when contrasted with sorghum (Vadez *et al.*, 2012). Subsequently, a large number of individuals rely upon this yield for their dietary necessities and business. Also, it has similarly been for quite a while known as a potential biofuel crop (Wu *et al.*, 2005; Lata, 2015). It could be a key feed source in a agrarian transformation in dry districts as it is a tropical plant having the C₄ photosynthetic pathway and resilience to dry season (Santos *et al.*, 2015). Therefore, pearl millet is a focal segment of the food security of the country's poor people in dry regions. In addition, among all cereals, it is the least expensive well spring of energy, protein, iron and zinc. These characteristics make pearl millet the significant supporter of protein, iron, and zinc admission in the areas where it is developed (Vadez *et al.*, 2012).

Grains of pearl millet are basically utilized for human consumption as different food and dry stover of pearl millet a premise of proportion for an enormous ox-like populace that is viewed as the most basic segment of giving strength in the risk-prone harvest domesticated animals cultivating framework in water-restricted districts (Yadav *et al.*, 2017). Critical bit of pearl millet grain is likewise utilized for non-food purposes, for example, poultry feed, steers feed and liquor extraction.

Pearl millet has narrowed production imperatives including different biotic and abiotic burdens, among which pearl millet

blast caused by *Magnaporthe grisea*, an as copycat parasite is a significant contemporary illness in the nation. Lately, it is turning into a genuine danger to pearl millet crop in India and around the world. Owing to the expansion in harmful races of microbe, impact sickness the executives systems appeared to be restricted. In India, this disease has been inconsistently seen on high yielding cultivars from 1953 onwards in the pearl millet developing areas having a place with Northern pieces of India. The disease occurrence information from 2002-2016 demonstrated that the illness is getting increasingly far and wide (AICRP Annual report, 2019).

Blast disease of *Pennisetum glaucum* is the leading vital devastating diseases limiting bulrush millet productivity. The DNA-based marker appliances encourage higher comprehension of the legacy and articulation of blast disease for ordinary crop improvement applications, PCR-viable markers upheld microsatellites or (SSRs) square measure ordinarily thought about the principal worthy. SSRs usually single-locus markers, that square measure typically co-dominantly heredity and characterised by hyper variability, abundance and reproducibility (Tripathi and Khare, 2016; Tripathi *et al.*, 2019; Bhawar *et al.*, 2019; Pramanik *et al.*, 2019; Baghel *et al.*, 2020; Mishra *et al.*, 2020; Shyam *et al.*, 2020; Upadhyay *et al.*, 2020; Verma *et al.*, 2021). Microsatellite markers are promising tool for in-depth investigations of genetic diversity of pearl millet (Kapila *et al.*, 2008; Thudi *et al.*, 2010; Kiranbabu *et al.*, 2013; Gupta *et al.*, 2015; Goud *et al.*, 2015; Sanghani *et al.*, 2018; Ambawat *et al.*, 2020). Consequently, the present investigation was carried out with the objectives to perform disease indexing at field level and SSR markers based screening against blast of pearl millet genotypes.

Materials and Methods

A total of forty-eight pearl millet germplasm lines registered in India were grown in field (Table 1) with divergent reactions to blast disease *viz.*: susceptible, tolerant and resistant. The seeds were obtained from All India Coordinated Research Project on pearl millet, College of Agriculture, Gwalior, RVSKVV, Gwalior, M.P., India. The field experiment was conducted at the experimental field, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior (M.P.) during Kharif 2019-20 and the laboratory work have been carried out at the Plant Molecular Biology & Biochemical Laboratories, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, India during July 2019 to March 2020.

The experimental material has been monitored in randomized block design (RBD) with two replications. Screening for resistance to blast has been done under natural field epiphytotic conditions as per method suggested by Wilson *et al.*, (1990) which has been standardized at ICRISAT. In this method test lines have been grown in the central four rows and a highly susceptible line on the first row and every fifth rows as infector/indicator rows.

Inoculate was sprayed at pre-tillering and/or pre-flowering stages with aqueous spore suspension. High humidity (>90% RH) was provided by operating sprinkler irrigation twice a day for 30-60 min each in the morning (between 10.00 a.m. and 11.00 a.m.) and in the afternoon (between 5.00 p.m. and 6.0 p.m.) on rain-free days. Disease severity was recorded at the hard-dough stage using a progressive 1-9 scale as developed for rice blast at International Rice Research Institute.

The disease severity rating scale has been modified to classify lines/plants into different disease reaction classes.

Apart from field screening, a total of 5 gene-specific Simple Sequence Repeats Markers (SSR) was employed for screening of pearl millet genotypes against blast (Babu *et al.*, 2012) and presented in Table 2. High quality genomic DNA was isolated from young and fresh leaves (8-10 days old) by using Cetyl trimethyl ammonium bromide (CTAB) as per Doyle and Doyle (1987) method with required modifications as suggested by Tiwari *et al.*, (2017). Genomic DNA was quantified using Nanodrop spectrophotometer. The qualitative analysis of genomic DNA was executed by agarose gel electrophoresis. The amplified products generated from molecular markers with PCR reaction were resolved on agarose gel. Each amplification product was considered as SSR bands were scored across all samples. The scoring was done based on banding pattern using standard size ladder. The major allelic frequency, polymorphism information content and genetic distance-based clustering was performed with Unweighted Pair Group Method for Arithmetic Average (UPGMA) tree using power Marker v3.25 software.

Results and Discussion

Field evaluation

A total of 48 pearl millet genotypes registered in India were grown in field were screened for resistance to blast has been done under natural field epiphytotic conditions according to method standardized at ICRISAT by Wilson *et al.*, (1990). In field screening, out of 48 genotypes 5 *viz.*, P343, IP205, THAK1827, THAK0201 and THAK 0285 were found highly resistance, 5 were resistance *namely*: IP315, IP368, IP378, IP114 and IP116, 20 moderate resistance *i.e.*, IP346, IP387, IP358,

IP302, IP307, IP342, IP338, IP354, IP384, IP353, IP382, IP368, IP378, IP327, IP280, IP107, IP109, IP118, IP119 and IP120, while 8 susceptible *viz.*, P370, IP343, IP300, IP345, IP355, IP350, IP310 and IP308 and 10 highly susceptible including IP351, IP373, IP375, IP347, IP325, IP344, IP359, IP376, IP324 and IP322 (Table 2; Fig.1) disease severity was documented at the hard-dough stage using a progressive 1-9 scale as developed for blast disease at International Rice Research Institute and screening techniques for pearl millet diseases described by Thakur *et al.*, (2011). Likewise, Gupta *et al.*, (2011) recorded the disease severity using same 1–9 progressive scale as mentioned for field screening. Sharma *et al.*, (2013) surveyed in the four major pearl-millet growing states in India *viz.*, Rajasthan, Haryana, Maharashtra and Uttar Pradesh for the prevalence of pearl millet blast. Blast severity was recorded using a 1 to 9 progressive scale and from the mini-core collection identified 32 germplasm accessions having resistance to at least one of the five pathotypes of *M. grisea* in India. Most of these accessions (21) originated in

India; therefore, germplasm accessions collected from different locations of India seem to be potential sources of blast resistance and could be evaluated against different pathotypes of *M. grisea* to identify additional sources of blast resistance. In our study out of 48 genotypes 5 were found highly resistance, 5 were resistance, 20 moderate resistance, 8 susceptible and 10 highly susceptible. Similarly, in recent study, Sharma *et al.*, (2019) identified possible diverse sources of blast and rust resistance in 305 accessions of *Pennisetum violaceum*, a wild relative of pearl millet, were screened against five pathotype-isolates of *M. grisea* and a local isolate of *P. substriata* var. *indica* collected from ICRISAT Farm, Patancheru, Hyderabad, India. Based on the mean blast score 1 to 9 scale, 17 accessions *viz.*, IP 21525, 21531, 21536, 21540, 21594, 21610, 21640, 21706, 21711, 21716, 21719, 21720, 21721, 21724, 21987, 21988 and 22160 were found to be resistant (score ≤ 3.0) against all five pathotypes, and 24 accessions were resistant to four pathotypes of *M. grisea*.

Table.1 List of genotypes used in the study

S. No.	Genotype						
1.	IP 315	13.	IP 358	25.	IP 350	37.	IP 109
2.	IP 346	14.	IP 302	26.	IP 310	38.	IP 114
3.	IP 370	15.	IP 307	27.	IP 376	39.	IP 116
4.	IP 351	16.	IP 342	28.	IP 324	40.	IP 118
5.	IP 373	17.	IP 345	29.	IP 332	41.	IP 119
6.	IP 387	18.	IP 355	30.	IP 308	42.	IP 120
7.	IP 363	19.	IP 344	31.	IP 382	43.	IP 201
8.	IP 343	20.	IP 359	32.	IP 368	44.	IP 203
9.	IP 375	21.	IP 338	33.	IP 378	45.	IP 205
10.	IP 347	22.	IP 354	34.	IP 327	46.	THAK 1827
11.	IP 325	23.	IP 384	35.	IP 280	47.	THAK 0201
12.	IP 300	24.	IP 353	36.	IP 107	48.	THAK 0285

Table.2 The blast disease scoring of pearl millet genotypes

Rating scale	Symptoms and lesions	Disease reaction	Name of Genotypes	Numbers of genotypes
1	No lesion to small brown specks of pinhead size.	Highly resistant	IP343, IP205, THAK1827, THAK0201 and THAK 0285	05
2-3	Large brown specks. Small, roundish to slightly elongated, necrotic gray resistant. Spots, about 1-2 mm in diameter with a brown margin.	Resistant	IP315, IP368, IP378, IP114 and IP116	05
4-5	Typical blast lesions, elliptical, 1-2 cm long, usually confined to the area between main veins, covering <2% of the leaf area. Typical blast lesions covering <10% of the leaf area.	Moderately resistant	IP346, IP387, IP358, IP302, IP307, IP342, IP338, IP354, IP384, IP353, IP382, IP368, IP378, IP327, IP280, IP107, IP109, IP118, IP119 and IP120,	20
6-7	Typical blast lesions covering 10-25% of the leaf area. Typical blast lesions covering 26-50% of the leaf area.	Susceptible	P370, IP343, IP300, IP345, IP355, IP350, IP310 and IP308	08
8-9	Typical blast lesions covering 51-75% of the leaf area and many leaves dead. >75% leaf area covered with lesions and most leaves dead.	Highly Susceptible	IP351, IP373, IP375, IP347, IP325, IP344, IP359, IP376, IP322 and IP324	10

Table.3 List of Gene-specific SSR markers used for screening of pearl millet genotypes against blast

SSR markers	Forward	Reverse
FMBLESTSSR1	AAGATCGAAACAAGCAAAACA	GAAAGAGTATGTGTTGCTTG
FMBLESTSSR2	TGGAAACAAGCAAAAGATAC	GTATGTTTTGCTTGTTCGA
FMBLESTSSR3	AAGATCGAAACAAGCAAAAG	GAGTATCTTTGCTTGTTC
FMBLESTSSR4	GATGGGAGACAAGCCAA	ACCTTTTGCTTGTATGGATC
FMBLESTSSR5	AAGATCCATACAAGCAAAAG	TCTTTTGCTTGTTCGATCT

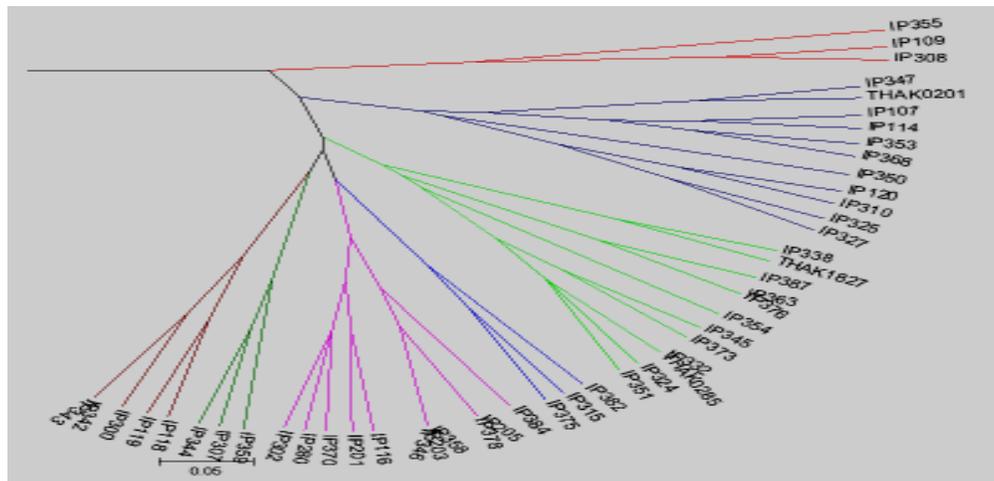
Table.4 Allele specific SSR markers presenting major allele frequency, number of alleles, gene diversity and Polymorphic Information Content (PIC)

Marker	Major Allele Frequency	Allele No.	Gene Diversity	PIC Value
FMBLESTSSR1	0.4792	4.0000	0.6302	0.5620
FMBLESTSSR2	0.5000	3.0000	0.5938	0.5112
FMBLESTSSR3	0.4792	3.0000	0.5564	0.4565
FMBLESTSSR4	0.4583	4.0000	0.5911	0.5048
FMBLESTSSR5	0.6042	3.0000	0.5304	0.4527
Mean	0.5042	3.4000	0.5804	0.4975

Fig.1 Blast symptoms on: (a & b) foliage; (c) sheath and (d) peduncle and panicle



Fig.2 Genetic relationships among 48 pearl millet genotypes are presented in molecular based UPGMA



Screening on the basis of gene-specific SSR molecular markers

In the present study, a total of 5 reported gene-based markers were taken for validation across forty-eight genotypes against blast disease including resistant check varieties viz., THAK1827, THAK0201 and THAK 0285. All 5 SSR primers were showed

polymorphism between tolerant and sensitive genotypes (Table 3).

A total of 17 alleles were detected with an average of 3.40 alleles per locus for different SSR markers. The gene diversity varied from 0.5304 to 0.6302 for SSR markers FMBLESTSSR5 and FMBLESTSSR1 respectively with an average of 0.5804 and

Polymorphic Information Content (PIC) values ranged between 0.4527 to 0.5620, for the markers FMBLESTSSR5 and FMBLESTSSR1 correspondingly with an average of 0.4975. The primer which showed highest gene diversity and PIC values was FMBLESTSSR1 while the lowest gene diversity and PIC values was documented for the primer FMBLESTSSR5. The major allele frequency varied in range of 0.4792 for primer FMBLESTSSR1 to 0.6042 for primer FMBLESTSSR5 with a mean value of 0.5042 (Table 4). Similarly Kapila *et al.*, (2008) studied genetic diversity among 70 maintainer lines of sub-Saharan and Indian origin by employing 34 primer pairs. A total of 213 alleles were detected with an average of 6.26 alleles per locus. Polymorphic information content (PIC) ranged from 0.05 to 0.96 with a mean value of 0.58 for the SSR loci. Mean PIC across the linkage groups and number of alleles in dinucleotide motifs varied significantly. The lowest PIC (0.239) for linkage group 6 indicated comparatively conserved nature of this linkage group. Genetic similarity estimates ranged from 0.05 to 0.73 with an average value of 0.29. Likewise, Thudi *et al.*, (2010) evaluated a set of 22 pearl millet inbred lines including the parents of eleven mapping populations, that were screened with 627 markers including 100 pearl millet genomic SSRs. The average PIC values and number of profiles (P) per polymorphic marker were: gSSRs (PIC = 0.62, P = 6.1). Kiranbabu *et al.*, (2013) also studied the genetic diversity among seven *M. grisea* isolates collected from major crop growing areas in India using 24 SSR molecular markers. Seventeen SSR markers were found to be polymorphic. Gupta *et al.*, (2015) carried out combined analysis of 379 hybrid parents using a set of highly polymorphic 28 SSRs markers and detected 12.7 alleles per locus. An average of 8.5 and 8.7 SSR alleles per locus were found in previously developed and current parents,

respectively, indicating marginal improvement in the levels of genetic diversity of hybrid parents in this programme. Subsequently Goud *et al.*, (2015) experimented cultural, pathogenic and molecular diversity in the *M. grisea* isolates infecting pearl millet and grouped them in five main clusters based on the results of molecular diversity study using URP markers. ICMR 06444 was found resistant at three locations *viz.*, Gwalior, Jamnagar and Patancheru and displayed moderate resistance at other three locations. In the present study, genotypes *viz.*, THAK1827, THAK0201 and THAK 0285 were found highly resistance at field as well as molecular level. Sanghani *et al.*, (2018) reported 55 SSR out of 100 SSR markers exhibited clear polymorphism between parental lines. Correspondingly Ambawat *et al.*, (2020) evaluated the diversity among 30 different released hybrids and varieties of pearl millet using 125 Simple Sequence Repeat (SSR) markers. Out of these, 61 polymorphic SSRs were reported giving 191 alleles with an average of 3.13 alleles per primer. Polymorphic Information Content (PIC) varied from 0.33 to 0.76 with an average of 0.55 PIC value.

Phylogenetic cluster analysis

The genetic relationships among pearl millet genotypes are presented in molecular based UPGMA tree. All the genotypes were grouped into 7 different clusters and among them cluster 2 and 3 are grouped with resistance against blast disease also having 3 check varieties (Fig. 2). Cluster 1 contained three genotypes including IP355, IP109 and IP308. Whereas Cluster 2 included eleven genotypes *viz.*, IP347, IP107, IP114, IP353, IP368, IP350, IP120, IP310, IP325, and IP327 along with a check variety THAK0201. Cluster 3 embraced twelve genotypes *i.e.*, IP338, IP387, IP363, IP376, IP354, IP345, IP373, IP332, IP324, IP351 and 2 check

varieties *viz.*, THAK827 and THAK0285 (Both check varieties having resistance against blast disease). They may have resistance gene for blast disease based on our field screening and molecular data interpretation. They represented higher yield groups also. Cluster 4 having three genotypes *viz.*, IP382, IP315 and IP351. While Cluster 5 included eleven genotypes *i.e.*, IP384, IP205, IP378, IP358, IP203, IP346, IP116, IP201, IP370, IP280 and IP302. Cluster 6 included three genotypes *namely*: IP359, IP307 and IP344 and Cluster 7 contained 5 genotypes including IP118, IP119, IP300, IP342 and IP343.

The genetic relationships among different pearl millet genotypes are presented in molecular based UPGMA tree. All the genotypes were grouped into 7 different clusters and among them cluster 2 and 3 is grouped with resistance for blast disease also having 3 check varieties. Similarly, Budak *et al.*, (2003) assessed the genetic diversity of 53 lines of millet by cluster analysis pair group method with arithmetic averages (UPGMA) showed two major and eight minor clusters, suggesting that the millet germplasm could readily be distinguished by UPGMA. Likewise, in a recent study conducted by Ambawat *et al.*, (2020), the cluster analysis based on SSR markers categorized the genotypes into four clusters with similarity coefficient ranging from 0.58 to 0.73.

In conclusion, during field screening, out of 48 genotypes 5 *viz.*, P343, IP205, THAK1827, THAK0201 and THAK0285 have been found highly resistance, 5 were resistance, 20 moderate resistance, 8 susceptible and 10 highly susceptible. In molecular analysis, 4 germplasm lines *viz.*, IP347, IP383, IP324 and IP351 including varieties, *namely*: THAK827, THAK0285 and THAK0201 have been found to be resistant against blast. These genotypes may

be used further in molecular breeding programmes to develop resistant/tolerant varieties against blast.

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