Research Article

Characterization Of Pearl Millet [*Pennisetum Glaucum* (L.) R Br.] Genotypes Against Downey Mildew Disease Employing Disease Indexing and ISSR Markers

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Abstract: Pearl millet (*Pennisetum glaucum* (L.) is nutritionally superior to principal cereals for instance rice or wheat owing to the presence of all the essential nutrients together with protein, minerals and energy. Crop losses due to plant diseases are economically important. Among the disease affecting, downy mildew, also known as green ear disease, is one of the most extensively spread and destructive disease of pearl millet, potentially resulting decline in production of crop in India and Western Africa. The present investigation was commenced with the objectives of disease indexing under field conditions and molecular characterization with gene-linked ISSR marker(s)of pearl millet germplasm line(s) against downy mildew. Under field condition, out of 48 germplasm lines, seven *i.e*., DMRBL2, ICHPR 18-4, ICMB-98222, ICMB-92888, DHLB-33, ICMB-02444 and ICMR-06222 were found highly resistant, 34 resistant, 4 susceptible and 3 were found to be highly susceptible. Among 5 downy mildew resistant gene-based ISSR markers the gene diversity varied from 0.6137 to 0.6997 for ISSR markers UBC-873 and UBC-807 respectively with an average of 0.6628. While Polymorphic Information Content (PIC) values ranged between 0.5317 to 0.6401 for the markers UBC-873 and UBC-807 correspondingly with a mean worth of 0.5932. Categorically, our findings of ISSR revealed existence of marker loci associated with the presence of disease resistance or susceptible genes in the set of pearl millet genotypes under investigation. The results may be further employed for marker assisted breeding for the improvement of crop against downy mildew disease.

Keywords: *Pearl millet, downy mildew, molecular markers, biotic stresses, resistance*

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1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) is a monocot species belongs to the family Poaceae and sub family Penicedae, having diploid genome (2n= 2x =14). It is locally known as Bajra, is also known as bulrush millet, cat tail or spiked millet (Verma *et al*., 2021a; Reddy *et al*., 2021). Spikelets of pearl millet are markedly protogynous, most of the styles having start to dry before pollen shed, so that the crop is mostly highly cross pollinated (Chelpuri *et al*., 2019; Choudhary *et al*., 2021a; Choudhary *et al*., 2021b; Choudhary *et al*., 2021c). It demonstrates highest level of tolerance to drought and heat, amongst domesticated cereals. It is nutritionally superior to major cereals for example rice or wheat owing to the presence of all the essential nutrients including protein, minerals and energy (Shinoj *et al*., 2006). It has around five times more calcium than wheat and rice congruently. The grain has higher level of protein content with balanced amino acids, CHO and fat which are important in human diet. Most species of millets are rich in iron, potassium and magnesium compared to rice and wheat (Davis *et al*., 2003). It is primarily grown for food and dry fodder therefore; the crop is gaining admiration as a health

food. A significant portion of pearl millet grain is also employed for non-food purposes for example poultry and cattle feeds and alcohol extraction (Basavaraj *et al*., 2010). Furthermore, vitamin and essential amino acid content of some common pearl millet species are higher than rice and wheat (Shobana *et al*., 2013).

In India, main states under pearl millet cultivation are Rajasthan, Maharashtra, Gujrat and Punjab. In the eastern side of the Western Ghats and in Tamil Nādu, it is grown as winter crop (Kumar *et al*., 2018; AICRPM, 2019). India is the greatest creator of pearl millet on the planet having a zone of 9.0 millionhawith production 11.70 million tons and productivity of 1300 $kgha^{-1}$ in 2021(PS&D Online updated on September 12, 2022).

In some of the hottest, driest regions of India and Africa, pearl millet is the only cereal that can be grown and playing a critical role in food security. In these hardest of environments, grain yield is severely limited by incidence of drought and diseases (Parihar*et al*., 2022). Crop losses due to plant diseases are economically significant. Among the diseases, affecting pearl millet, downy mildew, also known as green ear disease, is one of the most extensively spread and destructive disease of pearl millet, potentially resulting decline in production of pearl millet in India and Western Africa. It having great negative impact on Indian economy (Singh, 1995). It is caused by systemic infection by the obligate biotrophic pseudo-fungus *Sclerospora graminicola*, first reported on pearl millet in India by Butler *et al*. (1907). A major advantage of employing morpho-physiological, biochemical and molecular markers together for the screening the germplasm lines against disease at laboratory with field experiments may authenticate the results (Verma *et al*., 2021a). Molecular diversity analysis is becoming a method of choice for the improvement of agriculturally important plant genetic resources (Budak*et al*., 2003;Govindaraj*et al*., 2010;Sehgal et al., 2012; Singh et al., 2013; Sehgal *et al*., 2015; Kumar *et al*., 2017;Nehra*et al*., 2017;Baghel *et al*., 2020; Adlak *et al*., 2019; Pramanik *et al*., 2019; Bhawar *et al*., 2020; Shyam *et al*., 2020; Upadhyay*et al*., 2020; Mishra *et al*., 2021; Pramanik *et al*., 2021; Verma *et al*., 2021b; Yadav *et al*., 2021; Mandloi *et al*., 2022; Rathore *et al*., 2022; Singhal *et al*., 2022). The present investigation was commenced with the objectives of screening of pearl millet genotype(s)against downy mildew on the basis of disease indexing under field conditions andgene-linked ISSR marker(s).

2. **Material and Methods**

A total of 48 pearl millet germplasm lines (Table 1) were evaluated against downy mildew under sick in the kharif season of 2019experimental fieldat Research Farm, Department of Plant Molecular Biology &Biotechnologywith two replications in Randomized Block Design (RBD). The seeds were obtained from All India Coordinated Research Project (AICRP) on Pearl millet, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, India. The planting geometry (R x P) was kept 50 cm x 15 cm.At the time of sowing, a fine powder of oosporic material was added to the furrows. All standard agronomic practices were followed.Infector row 7042 S was sown and later test rows and indicator rows were sown. Total number of plants infected with downy mildew were recorded at 30 and 60 days after sowing.

Molecular Analysis work was performed at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Rajmata Vijayraje Scindia Agricultural University Gwalior, Madhya Pradesh, India.High quality genomic DNA was extracted from 8-10 days old young and fresh by employing CTAB method as proposed by Doyle and Doyle (1987) with some modifications as suggested by Tiwari et al. (2017). A total 5 gene-linked ISSR markers were employed for screening of pearl millet germplasm lines against downy mildew(Table 2).The PCR reaction mixture was prepared with Taq DNA polymerase, 10X PCR buffer, dNTPs, MgCl2, ISSR primer and divided equally (each of 23 µl) into PCR tubes. Two µl (25 ng/ µl) of genomic DNA of different lines were added in each tube. PCR amplification of genomic DNA was then performed in thermal cycler (Bio-Rad).

The amplified products generated from molecular markers with PCR reaction were resolved on agarose gel. Each amplification product was considered as ISSR bands were scored across all samples. The scoring was done based on banding pattern using standard size ladder. Data sheet was prepared to run in population structure and allele pattern A/A was used if band was in the upper side and pattern B/B was used if the band was in the lower side, in heterozygous condition banding pattern was A/B and in case of no amplification -/- was used.

The Polymorphism Information Content (PIC) was calculated as, where n is the number of band positions analysed in the set of accessions and P*I is the frequency of 1th allele. Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02 (Rohlf, 1993). The SIMQUAL programme was used to calculate the DICE coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on DICE coefficient.

3. **Results And Discussion**

3.1 Disease assessment and screening

Since downy mildew disease results in large output losses in the pearl millet it is very important economically for India and many other nations in Africa. A total of 48 pearl millet germplasm lines registered in India were grown under field conditions were screened for resistance to downy mildew has generally been done under natural field epiphytotic conditions according to method standardized at ICRISAT. Out of 48 germplasm lines, seven i.e., DMRBL 2, ICHPR 18-4, ICMB-98222, ICMB-92888, DHLB-33, ICMB-02444 and ICMR-06222 were found highly resistant, 34 resistant, 4 susceptible and 3 were highly susceptible (Table 1). Similar to the present study, field level screening of pearl millet for identification of downy mildew resistant and susceptible genotypes have been performed by various researchers (Kumar et al. 2010; Yadav et al. 2014; Gupta et al. 2015). Likewise, Sharma et al. (2007) evaluated in a sick plot using the field screening technique and reported that the mean downy mildew incidence on genotype 7042 S (indicator row) was more than 90% after 30 and 60 DAP, which indicated the uniform and good spread of the disease. Of the 147 pearl millet germplasm lines screened, 25 were found highly resistant, 32 resistant, 52 susceptible and 38 highly susceptible to downy mildew. During their analysis, among 25 highly resistant lines, ten lines viz., IP9, IP55, IP104, IP253, IP262, IP336, IP346, IP498, IP545 and IP558 were completely free from downy mildew infection at both the growing stages i. e., 30 and 60 DAP in both the years of testing (2005 and 2006). In our study, genotypes viz., IP244, IP280, IP206, IP234, IP216, IP251, IP254 and IP211 germplasm lines were found resistance against downy mildew. Similarly, Saritha et al. (2017) reported various control

entries 7042(S) showed 95.22-98.6% DMI and genotype IP18292 was found to possess 88.83-94.28% DMI across all Indian isolates of pathogen. Another susceptible control entry namely: 843B exhibited DMI values ranging from 94.25% to 97.75% across these Indian pathogen populations. A very highly resistant reaction was observed for resistant control 843-22B in screens against all three Indian pathogen populations (DMI values ranges from 0% to 4.13 %). Genotype ICMP 451 exhibited 84.37 - 96.18 % DMI against Sg445 and Sg519 except with Sg526 was showed 54.33 DMI%. In our study, we also found 93.8. % DMI for the genotype 7042(S).

Table 1 : Pearl millet germplasm having disease incidence

S.No.	Genotype	DM incidence (%)	Reactions
1	IP 224	5.7	R
2	IP 201	5.8	R
3	IP 228	10.6	S
4	IP 226	11.7	S
5	IP 225	13.5	S
6	IP 244	7.4	R
7	IP 280	6.4	R
8	IP 206	6.3	R
9	IP 234	7.7	R
10	IP 216	8.6	R
11	IP 251	6.9	R
12	IP 254	7.4	R
13	IP 211	8.3	R
14	IP 231	26.7	HS
15	IP 209	5.7	R
16	IP 229	6.8	R
17	IP 203	7.4	R
18	IP 235	5.7	R
19	IP 205	5.8	R
20	IP 232	5.8	R
21	IP 227	6.8	R
22	IP 202	7.9	R
23	IP 245	8.2	R
24	IP 281	27.8	HS
25	IP 293	5.8	R
26	IP 286	8.4	R
27	IP 247	8.7	R
28	IP 266	7.5	R
29	IP 298	5.9	R
30	IP 249	6.6	R
31	IP 258	6.7	R

32	IP 215	7.1	R
33	IP 212	12.4	S
34	IP 232	6.9	R
35	IP 223	8.5	R
36	852 B	8.1	R
37	7042 S	93.8	HS
38	DMRBL 2	1.4	HR
39	ICHPR 18-4	1.8	HR
40	ICMB-98222	2.7	HR
41	ICMB-92888	3.7	HR
42	JMSB 20175	11.6	S
43	DHLB-33	3.8	HR
44	ICMB-02444	2.7	HR
45	ICMR-06222	3.5	HR
46	BRRL-3	6.9	R
47	THAK 1827	7.2	R
48	THAK 0201	6.6	R

3.2 Validation of gene-based ISSR markers and screening against downy mildew

In present investigation, a set of 5 downy mildew resistance gene-based ISSR markers were employed for screening the 48 germplasm lines against downy mildew disease. A total of 17 bands were identified with an average of 3.40 alleles per locus for different ISSR markers (Table 2). Likewise, Animasaun *et al*. (2015) reported 48 loci consisting of 410 bands were generated with 56.25% polymorphism. The gene diversity varied between 0.6137 to 0.6997 for ISSR markers UBC-873 and UBC-807 correspondingly with an average of 0.6628. Gene diversity and Polymorphic Information Content (PIC) values varied from 0.5317 to 0.6401, for the markers UBC-873 and UBC-807 correspondingly with an average of 0.5932. The primer which demonstrated highest gene diversity and PIC values was UBC-807 while the lowest gene diversity and PIC values was documented for the primer UBC-873. The major allele frequency varied in range 0.3542 (UBC-807) to 0.4375 (UBC-8735) with a mean worth of 0.3917. Likewise, Marczewski (2001) reported ISSR markers UBC-811660 and UBC-811950 linked to Ns gene and the linkage distance were found 2.6 and 6.6 Cm, respectively. Molecular marker UBC-811660 showed high accuracy for detection of PVS resistance. Marker UBC-811660 could be a powerful tool for detection of germplasm lines carrying the Ns gene in breeding programme. Similarly, Mahatma *et al*. (2010) reported that some ISSR markers were only present in resistant germplasm lines *i.e*., UBC-825, UBC-827 and UBC-857. The single unique band was present only in susceptible germplasm of pearl millet. In accordance with our study, Zhang *et al*. (2004) also found that UBC 812 marker was linked with leaf rust resistance genes in wheat. Categorically, our findings of ISSR revealed existence of marker loci associated with the presence of disease resistance or susceptible genes in the set of pearl millet genotypes under investigation. The results

can be further applied for marker assisted breeding for the improvement of pearl millet crop. However, in a previous study, ISSR markers have been applied effectively in common bean (*Phaseolus vulgaris*) for the construction of linkage map consisting resistance to bacterial, fungal and viral pathogen of bean (Miklas *et al*. 2000). Similarly, Purnhauser *et al*. (2000) also used ISSR markers for identification of leaf rust resistant gene in common wheat.

Downy mildew resistance in pearl millet is quantitatively inherited, highly heritable, and responsive to selection (Angarawai *et al.* 2008). Molecular markers based selection has demonstrated its value in accelerating the process of precision breeding to create agriculturally significant varieties for disease resistance and a number of other features (Tripathi *et al.* 2022). Among different molecular markers ISSR have been used to select disease resistant genotypes. In this aspect, Jogaiah et al. (2014) used ISSR markers for the development of sequence characterized amplified region (SCAR) as a molecular screening tool to identify downy mildew resistance source in pearl millet. In their study, out of 27 ISSR markers used to detect polymorphism among the pearl millet genotypes ICMR-01007 (P1) and ICMR-01004 (P2) and their populations (F1 and F2), only one primer pair produced polymorphic bands on ICMR-01004 with a 1.4 kb size. The 1.4 kb band from the PCR amplification was found to be closely connected to the downy mildew resistant gene in ICMR-01004. The proposed SCAR primer (SCAR ISSR 863) was created after this band was cloned, sequenced, and analysed. The SCAR ISSR 863 marker was connected to the downy mildew resistance linkage group (2) at a genetic distance of 0.72 cM, according to segregant analysis of their F2 offspring.

Crop breeding and genetic improvement aims to increase genetic gain, which is the performance improvement brought about by artificial selection across generations. In the age of molecular breeding, phenotypic selection and other traditional breeding methods have evolved into molecular methods to increase genetic gain (Asati et al. 2022). Marker assisted selection stands out as a thorough method that gives breeders a useful instrument to put their expertise to use. It has been used to create lines resistant to biotic stress in pearl millet, enhancing overall genetic gain (Singh and Nara 2022).

Table 2: Allele specific ISSR markers presenting major allele frequency, number of alleles, gene diversity and Polymorphic Information Content (PIC)

Marker	Primer Sequence	Major Allele Frequency	Allele No	Gene Diversity	PIC
UBC-807	5′-(AG)8T-3′	0.3542	4	0.6997	0.6401
UBC-810	5′-(GA)8T-3′	0.4167	3	0.6424	0.5663
UBC-825	5′-(AC)8T-3′	0.3750	3	0.6632	0.5891
UBC-827	5′-(AC)8G-3′	0.3750	4	0.6953	0.6389
UBC-873	5′-(AC)8TG-3′	0.4375	3	0.6137	0.5317
Mean		0.3917	3.4	0.6628	0.5932

The genetic relationships among pearl millet germplasms are presented in molecular based UPGMA tree. All the germplasms were grouped into 6 different clusters and among them cluster 4, cluster 5 and cluster 6 was grouped with resistance germplasm lines against downy mildew disease also having 4 check varieties (Fig.1). Mahatma *et al*. (2010) also

suggested that ISSR data showed two distinct clusters of resistant and susceptible germplasm lines. These results suggested that ISSR markers may be used for large scale screening of germplasm lines against disease reaction.

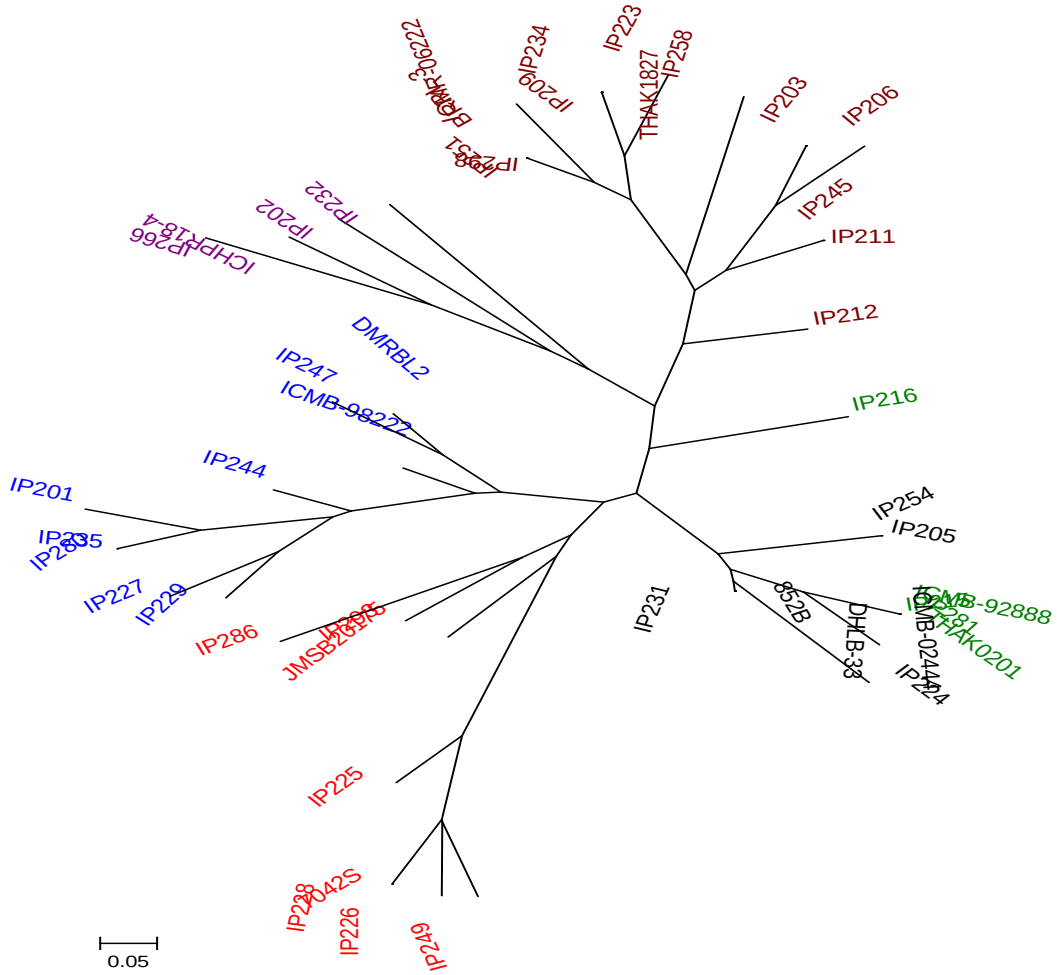

Fig.1: The genetic relationships tree among 48 pearl millet germplasm lines are presented in molecular based UPGMA

Conclusion

Cluster diagram analysis indicated that closely related germplasm lines based on yield attributing morphological traits are grouped together. It is also clear that genotype 7042S is grouped distinctively from other germplasm lines because it is highly susceptible and genotype IP224 is found most diverse from other as its similarity distance from other germplasm lines. Out of 48 germplasm lines, 7 viz., DMRBL2, ICHPR18-4, ICMB-98222, ICMB-92888, DHLB-33, ICMB-02444 and ICMR-06222 were found to be highly resistance, 34 resistance, 4 susceptible and 3 were highly resistance. All the germplasms were grouped into 6 different clusters and among them cluster 4, cluster 5 and cluster 6 was grouped with resistance for downy mildew disease also having check varieties.

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