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MOLECULAR ANALYSIS OF WORLD COLLECTION OF FINGER MILLET ACCESSIONS FOR BLAST DISEASE RESISTANCE USING FUNCTIONAL SSR MARKERS

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SUMMARY

The major limiting factor for production and productivity of finger millet is blast disease caused by *Magnaporthe grisea.* In the present study, most of the exotic genotypes showed highly resistant to moderately resistant reaction to neck blast and finger blast disease. Neck blast found to have significant and positive correlation with finger blast (0.612**), but a poor correlation was observed with leaf blast (0.08**). Fifty-eight genic SSRs were used for genetic diversity analysis of a global collection of 190 finger millet genotypes. Three SSR markers (RM23842, RM5963 and RM262) were found to be most highly polymorphic loci for differentiating the 190 global collections of finger millet genotypes. The SSR marker RM5963 containing the trimer repeat motif CAG showed highest PIC values, followed by tetra- (RM23842) and di- (RM262) repeat motif containing SSRs. The 58 genic SSR loci of blast disease grouped the 190 finger millet genotypes into 4 major clusters based on their blast disease response by phylogenetic clustering as revealed by Power Marker software. The average gene diversity existing among all the inbred lines was relatively high (49%), indicating existence of high levels of polymorphisms among the finger millet. Among the genotypes of NW Himalayan region of India, VHC3997 and VHC3930 found highly resistant to neck blast which can be used as donor parents for production of blast resistant finger millet varieties.

Keywords: Finger millet, blast disease, comparative genomics, EST-SSR markers, diversity, power marker

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INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn.) belongs to the family Poaceae, genus *Eleusine*. Finger millet is an important food crop cultivated widely in the arid and semi-arid regions of the world, especially in East Africa, India and other Asian countries including Sri Lanka and China. The crop is grown mainly by subsistence farmers and serves as a food security crop because of its high nutritional value and excellent storage qualities (Dida *et al.,* 2007). One of the major limiting factors for production and productivity of finger millet crop is blast disease caused by *Magnaporthe grisea*. This disease has been identified as the major constraint to finger millet production in Eastern Africa, and India since most landraces and a

number of other genotypes are highly susceptible (Mgonja *et al.,* 2007).

The development of modern plant breeding techniques has greatly facilitated the wider use of a wealth of diversity from many sources including landraces. Molecular markers have been applied in quantification of genetic diversity, genotype identification, gene mapping, association mapping and marker assisted selection (MAS). Assessment of the extent of genetic variation in finger millet has been carried out since the establishment of molecular biology techniques (Babu *et al.,* 2007; Kumar *et al.,* 2013). Expressed sequence tag (EST) projects have generated a vast amount of publicly available sequence data from plant species; these data can be mined for simple sequence repeats (SSRs). The EST databases have become particularly attractive resources for such *in silico* mining, as was demonstrated in citrus (Chen *et al.,* 2006), and particularly in cereals (Babu *et al.,* 2014a, 2014b, 2014c and 2014d), which can be further effectively being used in diversity studies, linkage map construction, QTL mapping studies (Senthilvel *et al.,* 2008) and marker assisted breeding programs for disease resistance.

Comparative genomics is a powerful tool for genome analysis, with the objective of understanding the detailed process of evolution at the macro level and of translating DNA sequence data into proteins of known functions. Srinivasachary *et al.* (2007) found high synteny (85%) between rice and finger millet genome.The availability of plenty of land races, diverse germplasm and gene sequence information in public databases, comparative genomics and molecular biology tools made it possible to characterize several important traits like blast resistance in neglected crops, where genome sequence is not available like finger millet. Knowledge of genetic variation present among the selected finger millet genotypes for blast resistance will pave the way for genetic mapping studies for the identification of genes for blast disease. With this aim, this study was conducted on a global collection of 190 finger millet accessions belongs to different parts of the world collection for genetic diversity analysis of finger millet genotypes for blast resistance using functional SSRs.

MATERIALS AND METHODS

Plant materials

In this study, a total of 190 finger millet accessions belong to different parts of the world including 8 countries of African continent (Zimbabwe, Kenya, Maldives, Uganda, Malawi, Senegal, Nigeria, and Zambia), 2 countries of South Asia (India and Nepal) and Germany were used. The genetic material of finger millet obtained from different sources, which included 84 finger millet accessions from the ICRISAT mini-core gene bank, ICRISAT, Hyderabad; 64 accessions from G.B.P.U.A &T, Pantnagar; 42 accessions from VPKAS, Almora was obtained (Table 1).

Field screening for blast disease severity assessment

The 190 finger millet accessions along with checks (VR 708, and RAU 8) were evaluated in the finger millet blast nursery at VPKAS, Almora, Uttarakhand, India. The blast disease severity assessment was done as per the earlier reports (Nagaraja *et al.,* 2007).

DNA extraction and quantification

The seeds were grown in pots under natural conditions. DNA was isolated from 10-day-old seedlings using the protocol developed by Murray and Thomson (1980). After extraction, 1 µl of DNA sample for all accessions was loaded in 0.8 % agarose gels. Uncut lambda DNA was loaded as a control to assess the quality and the quantity of DNA. Based on uncut lambda DNA standards, DNA samples were normalized to a uniform concentration (25 ng/ µl) and used for SSR genotyping.

SSR amplification and data analysis

The polymerase chain reaction (PCR) was performed in 20 µL reaction volume containing 2 µL 10x buffer having 15 mM MgCl2, 0.2 µM of each forward and reverse primer, 2 µL of 2 mM dNTPs, 0.2 µL of 0.2 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25 ng of template DNA. The PCR reactions are, initial

denaturation of 5 min at 94 °C followed by 40 cycles of 1 min at 94 °C, 1.5 min at different annealing temperatures for different primers, 2.0 min at 72 °C, and a final extension of 7 min at 72 °C, and hold at 4 °C. The PCR products were fractioned on 3.5% Super Fine Resolution (SFR) agarose gel. The electrophoresis was held at 100 volts for 3 h at room temperature. Gels were stained with ethidium bromide and visualized using Bio Imaging System (SynGene, USA) and the primers that amplified and showed detectable length polymorphism were identified. The data set of SSR loci on 190 accessions were used for diversity analysis using Power Marker V3.0 (Liu and Muse, 2005).

RESULTS AND DISCUSSION

Phenotyping of finger millet genotypes for blast resistance and their correlation

The finger millet genotypes were grouped into 4 groups (HR; R; MR-MS; S-HS) based on the disease score for neck blast. Based on the mean neck blast severity, 94 finger millet accessions were highly resistant (HR)/ resistant (R), 78 were resistant to moderately resistant (MR), 15 genotypes were susceptible (S) and 3 genotypes were highly susceptible (HS) compared to the susceptible check VR708 and resistant check RAU 8 (Table 2). Among the genotypes of NW Himalayan region of India, VHC3997 and VHC3930 found highly resistant to neck blast. Neck blast found to have significant and positive correlation with finger blast (0.612**), but a poor correlation was observed with leaf blast (0.08**). Kiranbabu *et al.,* (2012) also found highly significant correlation between neck blast and finger blast (0.92) and moderate correlation was observed with leaf blast (0.25) under artificial inoculations.

The finger millet genotypes were grouped into 4 groups (HR; R; MR-MS; S-HS) based on the disease score for finger blast disease resistance. Based on mean finger blast severity, 149 finger millet genotypes were found resistant to highly resistant, 32 were moderately resistant, while only 9 were found susceptible compared to the susceptible check VR708 and resistant check RAU 8 (Table 3).Among the genotypes from NW Himalayan region of India, VHC3997, VL324, and VHC3996 genotypes were highly resistant to finger blast. All the exotic genotypes found to be resistant to moderately resistant reaction to the finger blast disease. Recently, Kiranbabu *et al.* (2012) also found similar results, where they found 69 genotypes of ICRISAT mini-core collection were resistant, 4 moderately resistant to the finger blast disease. Based on mean leaf blast severity, only 13 genotypes (IE4121, IE2872, IE5066, IE2043, IE3317, IE2871, IE2217, IE3470, IE6154, IE4646, IE3470, GPHCPB11 and IE2589) found to show resistant to leaf blast, while the remaining all 177 genotypes were of susceptible to moderately resistant/susceptible to leaf blast. The genotypes showed resistant to leaf blast were also showed resistant reaction to leaf blast and neck blast. It was positively and significantly correlated with finger blast (0.227**).

Diversity analysis of finger millet genotypes for blast resistance using genic SSRs

Among the diseases, the blast disease caused by *M. griseae* is very prominent and affects the production, productivity of finger millet in the developing and under developed countries of the world. The average loss due to blast has been reported to be around 28-36% (Nagaraja *et al.,* 2007) and in certain cases yield loss could be as high as 80-90% (Rao, 1990). Hence, analysis of diversity among finger millet genotypes for blast resistance using the genic SSR markers is an important approach in the finger millet crop improvement program. The details of the genic SSR markers used in the present study were obtained from our earlier studies (Babu *et al.,* 2014a). The rice SSRs were obtained from Gramene website (www.gramene.org). The gel pattern of the SSR marker FMBLEST4 among 190 finger millet accessions was given (Figure 1).

Table 1. List of finger millet genotypes used in the study with origin and source of collection.

Genotype	Origin	Source
IE3391	Zimbambwe	ICRISAT mini-core
IE4491	Zimbambwe	ICRISAT mini-core
IE7320	Kenya	ICRISAT mini-core
IE4797	Maldives	ICRISAT mini-core
IE3077	India	ICRISAT mini-core
IE4121	Uganda	ICRISAT mini-core
IE4073	Uganda	ICRISAT mini-core
IE2710	Malawi	ICRISAT mini-core
IE2872	Zambia	ICRISAT mini-core
IE5066	Senegal	ICRISAT mini-core
IE4570	Zimbambwe	ICRISAT mini-core
VR708	India	VPKAS, Almora, India
IE5537	Nepal	ICRISAT mini-core
IE2043	ICRISAT	ICRISAT mini-core
IE3317	Zimbambwe	ICRISAT mini-core
IE2034	India	ICRISAT mini-core
IE2790	Malawi	ICRISAT mini-core
IE2871	Zambia	ICRISAT mini-core
IE5306	Zimbambwe	ICRISAT mini-core
IE4816	India	ICRISAT mini-core
IE4497	Zimbambwe	ICRISAT mini-core
IE6240	Zimbambwe	ICRISAT mini-core
IE6421	Uganda	ICRISAT mini-core
IE6165	Nepal	ICRISAT mini-core
IE4028	Uganda	ICRISAT mini-core
IE6221	Nepal	ICRISAT mini-core
IE6337	Zimbambwe	ICRISAT mini-core
IE6537	Nigeria	ICRISAT mini-core
IE6059	Nepal	ICRISAT mini-core
IE2821	Nepal	ICRISAT mini-core
IE4673	ICRISAT	ICRISAT mini-core
IE5367	Kenya	ICRISAT mini-core
IE3973	Uganda	ICRISAT mini-core
IE3104	India	ICRISAT mini-core
IE2572	Kenya	ICRISAT mini-core
IE2911	Zambia	ICRISAT mini-core
IE6350	Zimbambwe	ICRISAT mini-core
IE2430	Kenya	ICRISAT mini-core
IE5817	Nepal	ICRISAT mini-core
IE4734	India	ICRISAT mini-core
IE2042	India	ICRISAT mini-core
IE2217	India	ICRISAT mini-core
IE5091	Zimbambwe	ICRISAT mini-core
IE2619	Malawi	ICRISAT mini-core
IE2457	Kenya	ICRISAT mini-core
IE501	India	ICRISAT mini-core

IE2437	Kenya	ICRISAT mini-core
IE4622	Zimbambwe	ICRISAT mini-core
PRM1	India	ICRISAT mini-core
IE3475	India	ICRISAT mini-core
IE4795	Zimbambwe	ICRISAT mini-core
IE4565	Zimbambwe	ICRISAT mini-core
IE4757	India	ICRISAT mini-core
IE6154	Nepal	ICRISAT mini-core
IE3618	NA	ICRISAT mini-core
IE4646	Zimbambwe	ICRISAT mini-core
IE4671	India	ICRISAT mini-core
IE3470	India	ICRISAT mini-core
IE5106	Zimbambwe	ICRISAT mini-core
IE4057	Uganda	ICRISAT mini-core
IE7079	Kenya	ICRISAT mini-core
IE2957	Germany	ICRISAT mini-core
IE3945	Uganda	ICRISAT mini-core
IE5870	Nepal	ICRISAT mini-core
IE4545	Zimbambwe	ICRISAT mini-core
IE2312	India	ICRISAT mini-core
IE6294	Zimbambwe	ICRISAT mini-core
IE3392	Zimbambwe	ICRISAT mini-core
IE6473	Uganda	ICRISAT mini-core
IE6326	Zimbambwe	ICRISAT mini-core
IE7018	Kenya	ICRISAT mini-core
IE3045	India	ICRISAT mini-core
IE2296	India	ICRISAT mini-core
IE5201	India	ICRISAT mini-core
IE6082	Nepal	ICRISAT mini-core
IE3696	ICRISAT	ICRISAT mini-core
VHC3997	India	VPKAS, Almora, India
IE3698	Uganda	ICRISAT core
IE3699	Uganda	ICRISAT core
IE3700	ICRISAT	ICRISAT core
IE3701	ICRISAT	ICRISAT core
IE3702	ICRISAT	ICRISAT core
IE3703	ICRISAT	ICRISAT core
IE3770	ICRISAT	ICRISAT core
IE3769	ICRISAT	ICRISAT core
IE3704	ICRISAT	ICRISAT core
IE3797	ICRISAT	ICRISAT core
IE3768	ICRISAT	ICRISAT core
IE3791	Uganda	ICRISAT core
IE3793	ICRISAT	ICRISAT core
IE3775	ICRISAT	ICRISAT core
IE3794	ICRISAT	ICRISAT core
IE3772	ICRISAT	ICRISAT core
IE3771	ICRISAT	ICRISAT core

IE3795	Uganda	ICRISAT core
IE6514	ICRISAT	ICRISAT core
IE3712	ICRISAT	ICRISAT core
GE724	UP, India	GBPUA & T, Pantnagar
GE1621	KN, India	GBPUA & T, Pantnagar
GE1298	UP, India	GBPUA & T, Pantnagar
GE1583	UP, India	GBPUA & T, Pantnagar
GE2447	UP, India	GBPUA & T, Pantnagar
GE4692	Uganda	GBPUA & T, Pantnagar
GE4811	Malawi	GBPUA & T, Pantnagar
GE1899	UP, India	GBPUA & T, Pantnagar
GE1680	UP, India	GBPUA & T, Pantnagar
GE763	UP, India	GBPUA & T, Pantnagar
GE4440	Orissa, India	GBPUA & T, Pantnagar
GE1240	TamilNadu, India	GBPUA & T, Pantnagar
GE2238	TamilNadu, India	GBPUA & T, Pantnagar
GE1235	UP, India	GBPUA & T, Pantnagar
GE1936	UP, India	GBPUA & T, Pantnagar
GE1093	UP, India	GBPUA & T, Pantnagar
GE1146	UP, India	GBPUA & T, Pantnagar
GE2063	TamilNadu, India	GBPUA & T, Pantnagar
GE116	Madhya Pradesh, India	GBPUA & T, Pantnagar
GE2154	UP, India	GBPUA & T, Pantnagar
GE128	Bihar, India	GBPUA & T, Pantnagar
GE356	UP, India	GBPUA & T, Pantnagar
GE2136	TamilNadu, India	GBPUA & T, Pantnagar
GE384	AP, India	GBPUA & T, Pantnagar
GE390	TamilNadu, India	GBPUA & T, Pantnagar
GE3147	KN, India	GBPUA & T, Pantnagar
GE1537	UP, India	GBPUA & T, Pantnagar
GE619	TamilNadu, India	GBPUA & T, Pantnagar
GE514	UP, India	GBPUA & T, Pantnagar
GE4449	Orissa, India	GBPUA & T, Pantnagar
GE1437	UP, India	GBPUA & T, Pantnagar
GE2624	UP, India	GBPUA & T, Pantnagar
GE2471	UP, India	GBPUA & T, Pantnagar
GE4404	Bihar, India	GBPUA & T, Pantnagar
VHC3944	India	VPKAS, Almora, India
VHC3870	India	VPKAS, Almora, India
VHC3962	India	VPKAS, Almora, India
VHC3996	India	VPKAS, Almora, India
VHC3991	India	VPKAS, Almora, India
VHC3984	India	VPKAS, Almora, India
VHC3980	India	VPKAS, Almora, India
VHC3970	India	VPKAS, Almora, India
VHC3951	India	VPKAS, Almora, India
VHC3907	India	VPKAS, Almora, India
VHC3908	India	VPKAS, Almora, India

VHC3887	India	VPKAS, Almora, India
VHC3881	India	VPKAS, Almora, India
VHC3956	India	VPKAS, Almora, India
VHC3930	India	VPKAS, Almora, India
VHC3876	India	VPKAS, Almora, India
VHC3903	India	VPKAS, Almora, India
VHC3895	India	VPKAS, Almora, India
VHC3898	India	VPKAS, Almora, India
VHC3893	India	VPKAS, Almora, India
VHC4013	India	VPKAS, Almora, India
VHC3873	India	VPKAS, Almora, India
VHC3865	India	VPKAS, Almora, India
VHC3911	India	VPKAS, Almora, India
VHC3972	India	VPKAS, Almora, India
VHC3917	India	VPKAS, Almora, India
VHC3939	India	VPKAS, Almora, India
GE496	MP, India	GBPUA & T, Pantnagar
GE669	Africa	GBPUA & T, Pantnagar
GE796	NA	GBPUA & T, Pantnagar
RAU8	India	GBPUA & T, Pantnagar
GPU28	NA	GBPUA & T, Pantnagar
GPU48	NA	GBPUA & T, Pantnagar
KM252	NA	GBPUA & T, Pantnagar
VR708	India	VPKAS, Almora, India
VL149	India	VPKAS, Almora, India
VL324	India	VPKAS, Almora, India
VL333	India	VPKAS, Almora, India
VL201	India	VPKAS, Almora, India
VL204	India	VPKAS, Almora, India
GE5192	Uganda	UAS, Bangalore
VL315	India	VPKAS, Almora, India
GE4440	Orissa, India	UAS, Bangalore
GPHCPB1	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB2	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB4	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB5	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB10	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB11	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB13	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB20	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB25	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB26	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB27	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB29	Pantnagar, India	GBPUA & T, Pantnagar
GPHCPB30	Pantnagar, India	GBPUA & T, Pantnagar
IE2589	America	ICRISAT mini-core
IE3614	NA	ICRISAT mini-core
VHC3697	India	VPKAS, Almora, India

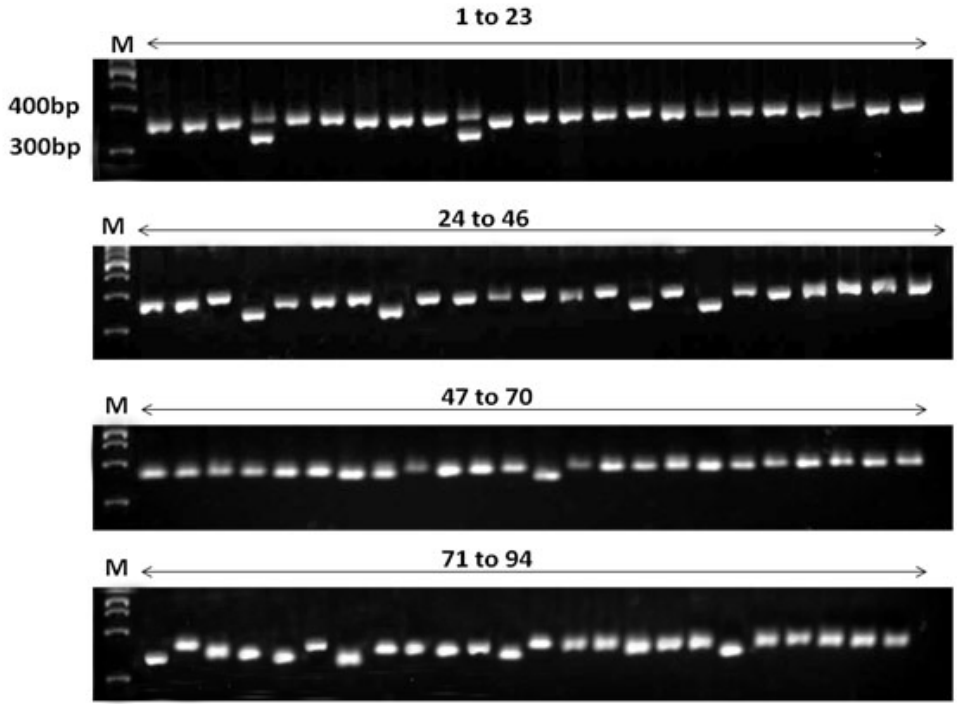
NA = not available

Table 2. The grouping of finger millet genotypes according to their response to neck blast disease.

Neck blast disease severity	Scale/ Rank	Finger millet genotypes
Highly resistant	0-1	IE3391, IE4491, IE7320, IE4797, IE3077, IE4121, IE4073, IE4570, IE5537, IE2043, IE3317, IE2034, IE2790, IE2871, IE5306, IE4816, IE4497, IE4028, IE6537, IE6059, IE5367, IE3973, IE2911, IE6350, IE2430, IE2619, IE2457, IE3475, IE4565, IE6154, IE4646, IE4671, IE5106, IE3945, IE6326, IE7018, IE2296, IE3696, IE3997, IE3698, IE3699, IE3700, IE3701, IE3770, IE3769, IE3704, IE3797, IE3791, IE3793, IE3775, IE3794, IE3772, GE1298, GE128, GE3147, GE1537, GE1437, VHC3930, GE669, RAU8, GPU48, IE3614
Resistant	2	IE2710, IE5066, IE6240, IE6421, IE6337, IE2821, IE4673, IE4734, IE2437, IE4622, IE4795, IE4757, IE3618, IE4057, IE2957, IE2312, IE6294, IE3392, IE6473, IE3045, IE3702, IE3703, IE3768, IE3771, IE3795, IE3721, GE4811, GE1240, GE2238, GE1235, GE1936, GE1146, GE2063, GE390, GE619, GE4449, VHC3991, VHC3984, VHC3908, VHC3881, VHC4013, VHC3972, GE796, GPU28, KM252, VL149, VL324, VL333, VL201, VL204, GE4440, GPHCPB11, IE2589
Moderately resistant to moderately susceptible	3	IE2872, IE6165, IE6221, IE2572, IE5817, IE2042, IE2217, IE5091, PRM1, IE3470, IE7079, IE5870, IE4545, IE5201, IE6514, GE724, GE2447, GE4692, GE1899, GE1680, GE763, GE4440, GE1093, GE116, GE2154, GE356, GE2136, GE384, GE554, GE2624, GE2471, GE4404, VHC3944, VHC3870, VHC3962, VHC3996, VHC3980, VHC3907, VHC3887, VHC3956, VHC3876, VHC3895, VHC3898, VHC3873, VHC3865, VHC3911, GE496, GE5192, GPHCPB2, GPHCPB4, GPHCPB10, GPHCPB13, GPHCPB25, GPHCPB29, GPHCPB30,
Susceptible to highly susceptible	4-5	IE3104, IE501, IE6082, GE1621, GE1583, VHC3970, VHC3951, VHC3903, VHC3893, VHC3917, VHC3939, VR708, VL315, GPHCPB1, GPHCPB5, GPHCPB20, GPHCPB26, GPHCPB27, VHC3697

Table 3. The grouping of finger millet genotypes according to their response finger blast disease.

Finger blast disease severity	Scale/ Rank	Finger millet genotypes
Highly resistant	0-1	IE4491, IE7320, IE4797, IE4073, IE5537, IE2043, IE3317, IE4497, IE4028, IE6165, IE2430, IE4646, IE4671, IE5106, IE3945, IE7018, IE3696, IE3997, IE3698, IE3699, IE3770, IE3769, IE3704, IE3797, IE3791, IE3793, IE3775, IE3794, GE1437, GE669, RAU8, GPU48, IE5066, IE6337, IE3470, IE7079, IE2312, IE6294, IE3045, IE3768, GE1936, GE2063, GE390, VHC3996, KM252, VL324
Resistant	2	IE3077, IE4121, IE2710, IE2872, IE4570, IE2034, IE2790, IE2871, IE5306, IE4816, IE6421, IE6221, IE6537, IE6059, IE2821, IE4673, IE5367, IE3973, IE2572, IE2911, IE6350, IE4734, IE2042, IE5091, IE2619, IE2457, IE2437, IE4622, PRM1, IE3475, IE4795, IE4565, IE4757, IE6154, IE3618, IE4057, IE2957, IE5870, IE4545, IE3392, IE6473, IE6326, IE2296, IE5201, IE3700, IE3701, IE3702, IE3703, IE3772, IE3795, IE6514, IE3721, GE724, GE1298, GE2447, GE4692, GE4811, GE1899, GE4440, GE2238, GE1235, GE128, GE3147, GE1537, GE619, GE4449, GE2624, GE4404, VHC3944, VHC3962, VHC3991, VHC3984, VHC3907, VHC3908, VHC3887, VHC3881, VHC3956, VHC3930, VHC3876, VHC3895, VHC3898, VHC3873, VHC3865, VHC3911, VHC3972, GE796, GPU28, VL149, VL333, VL201, VL204, GE5192, GE4440, GPHCPB2, GPHCPB10, GPHCPB11, GPHCPB29, GPHCPB30, IE2589, IE3614
Moderately resistant to moderately susceptible	3	IE3391, IE6240, IE3104, IE5817, IE2217, IE6082, GE1621, GE1583, GE1680, GE763, GE1240, GE1093, GE1146, GE116, GE2154, GE356, GE2136, GE384, GE554, GE2471, VHC3980, VHC3951, VHC3903, VHC3893, VHC4013, GE496, VL315, GPHCPB4, GPHCPB5, GPHCPB25, GPHCPB27, VHC3697
Susceptible to highly susceptible	4-5	IE501, VHC3970, VHC3939, VR708, GPHCPB1, GPHCPB20, GPHCPB26, VHC3697, VHC3870, GPHCPB13



(Lane M- 100bp marker, Lane 1 to 94- IE3391, IE4491, IE7320, IE4797, IE3077, IE4121, IE4073, IE2710, IE2872, IE5066, IE4570, IE5537, IE2043, IE3317, IE2034, IE2790, IE2871, IE5306, IE4816, IE4497, IE6240, IE6421, IE6165, IE4028, IE6221, IE6337, IE6537, IE6059, IE2821, IE4673, IE5367, IE3973, IE3104, IE2572, IE2911, IE6350, IE2430, IE5817, IE4734, IE2042, IE2217, IE5091, IE2619, IE2457, IE501, IE2437, IE4622, PRM1, IE3475, IE4795, IE4565, IE4757, IE6154, IE3618, IE4646, IE4671, IE3470, IE5106, IE4057, IE7079, IE2957, IE3945, IE5870, IE4545, IE2312, IE6294, IE3392, IE6473, IE6326, IE7018, IE3045, IE2296, IE5201, IE6082, IE3696, VHC3997, IE3698, IE3699, IE3700, IE3701, IE3702, IE3703, IE3770, IE3769, IE3704, IE3797, IE3768, IE3791, IE3793, IE3775, IE3794, IE3772, IE3771)

Figure 1. The molecular characterization of 94 finger millet genotypes with the blast specific genic SSR marker FMBLEST4**.**

Three SSR markers were found to be most highly polymorphic loci among the 190 global collections of finger millet genotypes. Among the polymorphic SSR loci, 9 loci had dimer repeat motifs, whereas 5 loci had trimer repeat motif. Six loci had penta- repeats, while 5 had hexa- repeat motif. The SSR loci RM5963 containing the trimer repeat motif CAG showed highest PIC values, followed by tetra- (RM23842) and di- (RM262) repeat motif containing SSRs. The mean PIC values of the SSR loci according to their repeat motifs showed that, tetra- repeats had highest PIC value followed by tri- repeat motifs. The di-, penta- and hexa- repeat motifs had nearly same PIC values. Reddy *et al.* (2012) also found similar results that tri- and tetra- repeat motifs had highest PIC value than the other repeat motifs. The SSR loci RM5963 (CAG) and RM23842 (AGAT) belonged to tri- and tetra- repeat motif also has maximum number of alleles (4). All the SSR markers containing penta- repeat motif had generated only 2 allele per locus. These results indicated that tri- and tetra- repeat motifs were more polymorphic and consisted of more PIC, gene diversity and allele number and were reported earlier in finger millet (Reddy *et al.*, 2012).

The genetic diversity analysis among the global collection of 190 finger millet genotypes for blast resistance were done using 58 genic SSRs present wide spread among all the chromosomes. The dendrogram was generated through UPGMA analysis of Power Marker V3.25 software.

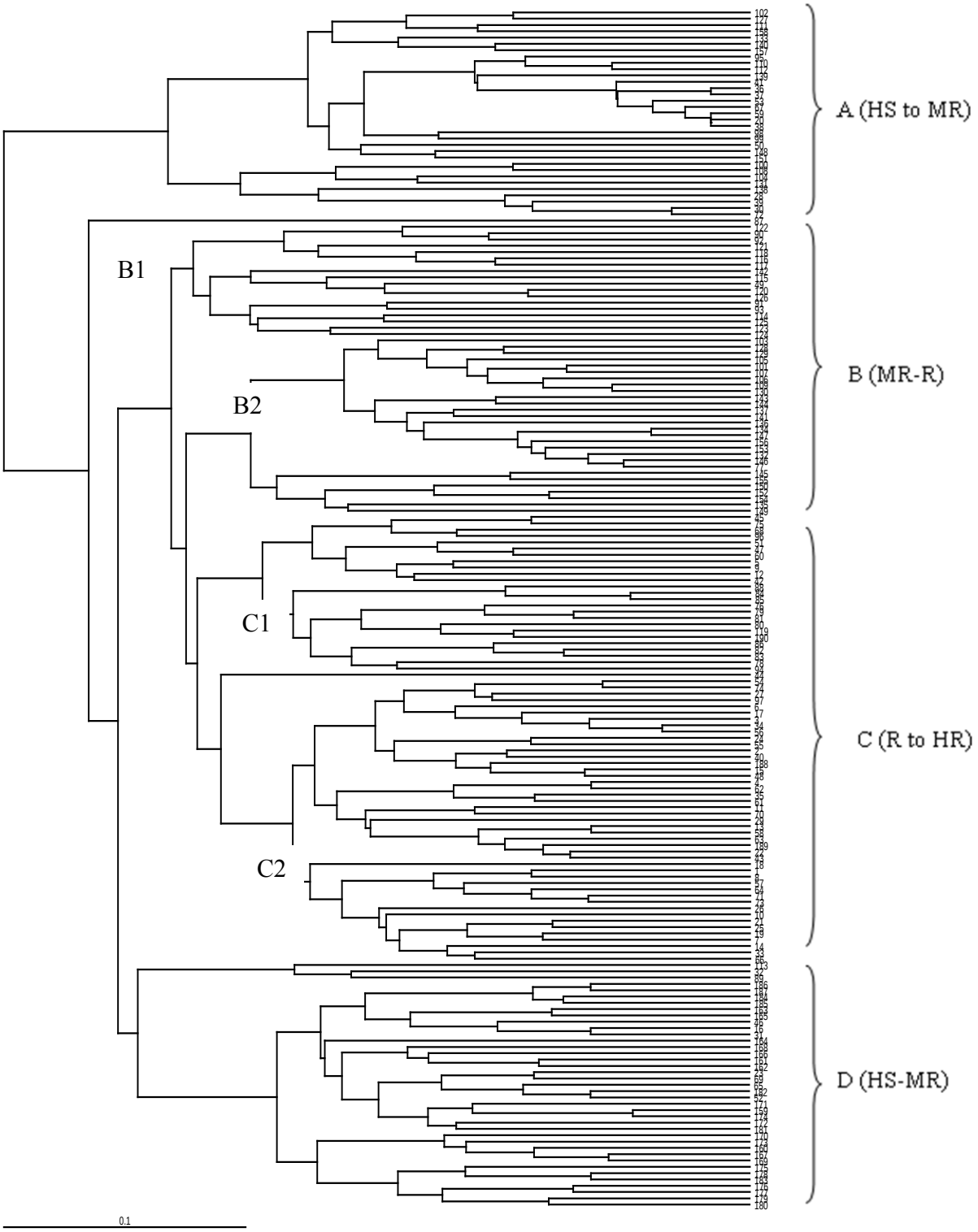


Figure 2. The dendrogram generated by power marker software for blast resistance using genic SSRs of finger millet (HS-Highly susceptible; MR-Moderately resistant; R- Resistant; HR- Highly resistant).

These 58 SSR loci grouped the 190 finger millet genotypes broadly based on their blast disease resistance into 4 major clusters (A, B, C and D) based on the UPGMA analysis of Power Marker V3.25 software (Figure 2).

The cluster 'A' comprised of genotypes that showed HS-MR response to blast, while cluster 'B' comprised of genotypes which were of MR-R type of blast resistance. The cluster 'C' consisted of mostly the resistant and highly resistant genotypes, while 'D' cluster comprised the genotypes that showed HS-MR type of response to blast. This grouping of the finger millet genotypes showed that the functional SSR markers could broadly able to differentiate the genotypes based on their response to blast disease. However, Dida *et al.* (2008) analyzed a set of 79 finger millet accessions using 45 SSR markers and were able to differentiate into 2 phylogenetic groups according to their geographic origin based on the power marker analysis. Similar results have been obtained by some worker like Nirgude *et al.* (2014) grouped 103 finger millet genotypes into 3 groups based on the protein content by using the functional SSR markers. Recently, Kumar *et al.* (2012) differentiated the finger millet genotypes based on the calcium content of the genotypes by using genic SSR markers. Kiranbabu *et al.* (2012) studied the resistance to blast in a mini core collection of finger millet germplasm and grouped the genotypes based on resistant, moderately resistant and susceptible genotypes which were in well congruence with our studies.

The HS-MR genotypes were clustered into 2 different sub clusters (A and D). This further grouping of the HS-MR genotypes may be due to base on their geographic origin. The cluster A genotypes were mostly from different parts of India, where as cluster D comprised genotypes of mostly from GBPUAT, Pantnagar, NW Himalayan region of India. This showed that genic markers were very effective in differentiating finger millet genotypes solely based on their blast disease score and then further grouping was done according to geographic origin. The average gene diversity existing among all the inbred lines were relatively high (49%), indicating existence of high levels of polymorphisms among the finger millet. These results are in close agreement with the findings reported among the cereal crops like maize inbreds using a SSR marker system. The cluster A comprised of 33 finger millet genotypes which had a range of response to the blast disease from highly susceptible to medium resistance or medium susceptible. The genotypes VHC3917, VHC3939, VHC3870, VHC3970, VHC3951, VHC3903, GE1621, and VHC3893 were highly susceptible to susceptible for the finger millet blast disease and were grouped under cluster A. These genotypes were clustered along with few MR/ MS genotypes like GE2447, IE2042, GE724, GE440, GE4404, VHC3980 and IE5817. All these genotypes were mostly of Indian origin and also an exotic finger millet genotype IE5817 (Nepal) was clustered. This may be due to that, the Nepal region geographically similar to NW Himalayan region of India. Also, most of the Indian genotypes were early flowering and hence they were susceptible to the blast disease, which was proved by earlier reports (Nagaraja *et al.,* 2007). A significant negative correlation was found between blast severity and days to flowering (DF) suggesting that early flowering accessions were more susceptible than the late ones (Mgonja *et al.,* 2007) and similar conclusions were made by Kiranbabu *et al.* (2013). For instance the accessions VHC3917, VHC3939, and VHC3970 were the earliest to flower (64-69 days) recorded highest neck blast severity and finger blast severity. Among the genotypes of VPKAS, Almora, the genotypes which showed HS and S reaction to blast were grouped under cluster A, while the genotypes that showed MR reaction to blast disease were grouped under cluster B. This indicated that, ability of our designed functional SSR markers were very effectively able to differentiate the genotypes from the same geographical origin into based on their blast resistance. The cluster A also consisted of few exceptions of exotic genotypes IE2911, IE6350 and IE2430 which were from Zambia, Zimbabwe and Kenya respectively and were resistant genotypes. This may be due to disease escape. Though these genotypes phenotypically appear as resistant, but genetically might be having the susceptible alleles and hence grouped with the susceptible genotypes.

The cluster B comprised of 48 genotypes that showed MR-R reaction to finger millet blast disease where mostly were of moderate resistance to the blast disease. Out of the 48 genotypes, most of the genotypes (37) showed MR to the blast disease, while 10 genotypes showed resistance to the blast disease. The sub cluster B2 comprised of mostly moderately resistant genotypes except the genotypes VHC3881, VHC3984, VHC3991, VHC3930 and VHC3972 which were resistant to blast disease. The cluster B1 consisted of both medium resistant and resistant genotypes. The resistant genotypes IE3797, GE390, GE128, VHC3908, GE2063, GE3147, GE619, IE3775, and IE3772 were all of Indian origin but belonged to different parts of the India like northern, southern and NW Himalayan regions of India. These results indicated that the functional SSR markers were even able to differentiate between MR and R genotypes effectively.

The cluster C comprised broadly the resistant genotypes that showed highly resistant to resistant reaction to the finger millet blast disease with some exceptions. All the genotypes under the cluster C were mostly belonged to exotic origin. This may be due to the fact that, all the exotic lines were late flowering genotypes and hence they were not susceptible to the blast disease and the virulent stages of the pathogen were not coincident with the susceptible stages of the host plant. Similar conclusions have been made by Kiranbabu *et al.* (2013) where they have analyzed the ICRISAT mini-core collection of finger millet genotypes and most of the genotypes were late flowering and were shown the resistant reaction to the blast disease based on the artificial screening. This showed that the blast screening by natural means and artificial means showed the nearly similar results and also it may be due to that the field chosen for blast screening nursery was known to be hot spot for blast disease of finger millet and rice. The cluster C further sub divided into 2 sub-clusters C1 and C2. The cluster C1 consisted of 25 genotypes which comprised of resistant genotypes along with few genotypes that were moderately resistant to the finger millet blast disease. There were 6 genotypes which were moderately resistant, IE6082 (Nepal), IE6514 (ICRISAT), VR708, IE2217 (India), IE2872 (Zambia), GE356 (India). All these genotypes were under sub cluster C1 which were separated from the remaining genotypes. The sub-cluster C2 consisted of 46 genotypes, where all were of resistant genotypes.

The cluster D consisted of 36 finger millet genotypes, which were having wide responses to the blast disease from HS to MR response. Under the cluster D, the susceptible genotypes, GPHCPB13, VL315, GPHCPB1, and GPHCPB5 were all grouped together and all showed the susceptible response to the finger millet blast disease. Medium resistant genotypes GPHCPB25, GPHCPB4, GPHCPB2, GPHCPB10, were under 1 cluster, whereas the genotypes GPHCPB29, and GPHCPB30 were under separate cluster and grouped together with GPHCPB 27 and GPHCPB 26 which were of susceptible, and highly susceptible respectively to the blast disease. The cluster D also contained few resistant genotypes like GE796, and VL324 which were clustered together and deviated from the other HS, S and MR genotypes. This showed that there was mixture of alleles responsible for resistance and hence grouped under the same cluster. Similarly Dida *et al.* (2008) also found a profound admixture between the Asian and African populations. Developing the improved blast resistant varieties with broad spectrum resistance activity is an important breeding goal in finger millet improvement (Mgonja *et al.*, 2007). Hence, in the present study the highly diverse genotypes that were highly resistant and highly susceptible genotypes can be effectively used in the fine mapping of genes responsible for blast resistance.

CONCLUSION

Thus this study is the first report assessing the genetic diversity among the finger millet genotypes using functional SSR markers for blast resistance among a diverse set of global collection of finger millet genotypes. Several genotypes belonged to different parts of the world were found to be resistant to all the 3 types of blast resistance both at field level and molecular level. The developed functional markers were able to differentiate the finger

millet genotypes into 4 groups based on their blast disease response, which showed that the developed markers were effective for blast disease resistance. The results showed that the resistant alleles were mostly present in the exotic genotypes rather than the Indian genotypes. The results found in the study will be very much useful for development of blast resistant varieties to the NW Himalayan region of India.

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