Plant Genetic Resources: Characterization and Utilization

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Molecular fingerprinting of highly resistant maize lines to turcicum leaf blight

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Research Article

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Abstract

The present study generates information related to the molecular divergence between turcicum leaf blight (TLB)-resistant and -susceptible lines. During molecular diversity studies, a total of 212 alleles were detected at 75 marker loci and ranged from two to five with an average of 2.83 alleles per locus. A direct correlation for the number of alleles and polymorphism information content (PIC) values was ascertained. For instance, marker phi123 produced high number of alleles (5) with PIC values of 0.77. Using the DARwin 6.0 programme, the UPGMA dendrogram grouped 40 maize inbreds into two distinct clusters, cluster-I (36 inbreds) and cluster-II (4 inbreds). Cluster-I contained two subclusters; the first subcluster contained 28 inbreds and the second subcluster contained eight inbreds whereas cluster-II contained four inbreds. This major cluster-II was further classified into two subclusters which contained two inbreds each. Most of the inbred lines except V-25 from cluster-II were highly resistant to TLB disease. These inbred lines can be used in crossing programmes to develop TLB-resistant hybrids by using divergent parents. In this study, allelic diversity and PIC values indicated a good efficiency of markers for studying the polymorphism level available in studied inbred lines. High level of diversity among the inbreds detected with simple sequence repeat markers indicated their suitability for the further breeding programme.

Introduction

Turcicum leaf blight (TLB), a significant foliar disease caused by *Exserohilum turcicum* (Pass.), can significantly reduce the yield of maize (*Zea mays* L.), the third-most important grain crop in the world (Leonard and Suggs, 1974; Jakhar *et al.*, 2022). Grain yield can be reduced up to 80% when TLB becomes severe (Tefferi *et al.*, 1996). Long, elliptical, brown or greyish green leaf lesions that begin on the lower leaves and eventually spread throughout the foliage are the characteristic features of the disease. Blighted leaves die too soon if the disease is not treated in early stages (Jakhar *et al.*, 2017; Keerthana *et al.*, 2023). Therefore, planting resistant cultivars is a widely accepted strategy that can reduce the rate at which diseases emerge (Keerthana *et al.*, 2023). Disease resistance is classified as either qualitative or quantitative. Qualitative disease resistance is exclusive to a certain race, but quantitative or polygenic disease resistance is typically non-specific to a particular race and is more common in the Indian subcontinent (St. Clair, 2010). The initial study on *E. turcicum* in several Indian races was carried out by Payak and Sharma (1985). Gowda *et al.* (1993) found the four maize differentials, *A-503HtN*, *H-4460Ht*, *H-4460Ht2* and *H-4460Ht3*, that include the numerous *Ht resistance* genes (*Ht* for *Helminthosporium turcicum)*. A longer incubation period and fewer, usually smaller disease lesions are characteristics of quantitative TLB resistance, which is durable (Brewster *et al.*, 1992; Smith and Kinsey, 1993; Abebe *et al.*, 2008; Gulzar *et al.*, 2018). Jakhar *et al.* (2021) screened 40 maize inbreds for the TLB resistance in Varanasi and Nagenahalli under artificially inoculated field conditions and observed that 10 inbreds *viz*., HKI-586, HUZM-53, CM-145, V-336, V-338, HKI-PC-8, HUZM-47, CM-104, CM-105 and CML-192 were resistant for the TLB disease. Similarly, Alemayehu *et al.* (2018) also evaluated the disease reaction of maize lines against common leaf rust and TLB under field conditions with artificial inoculation. The development of resistance against TLB disease will have a significant effect on the maize breeding programmes.

The presence of distinct genetic groups among maize inbreds is characterized to increase gene diversity that is helpful in optimizing hybrid vigour. Using molecular markers to create diversity among maize inbred lines is a valuable tool (Singh and Srivastava, 2017). Because it is highly polymorphic, repeatable and co-dominant in nature, the microsatellite marker is determined to be the most appropriate molecular marker among the many classes for screening TLB resistance (De Loose and Gheysen, 1995; Singh and Srivastava, 2017; Keerthana *et al.*, 2023). The microsatellite markers are useful in characterizing maize inbreds and in establishing distinct clusters based on genetic diversity and also useful in maize breeding programmes (Nikolic *et al.*, 2015). Likewise, based on UPGMA clustering method, the inbred lines were

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Table 1. List of 40 maize inbreds used in this study and their pedigree/source germplasm details, major characteristics along with TLB disease reaction

S. No.	Inbreds	Pedigree/source germplasm	Major characteristics	TLB disease reactions
1	Dhiari Local	VPKAS, Almora	Yellow kernels with flint texture and highly susceptible to turcicum leaf blight (TLB) disease	HS/S
2	V-341	Derivative of Mexico Acc No. 3136, Almora	Flint texture with early maturity, ear shape is conical and medium plant height	PR
3	HKI-586	CH 3, Karnal	Early dark green erect leaves and resistance to TLB disease	R
4	CM-141	Pool 33(Alm), Almora	Yellow kernel with flint texture and early maturity and leaf angle is narrow	PR
5	CML-118	SIYFs3#b1-7-b1-b1#b1, Mexico	Fine kernel texture with moderate resistance to TLB	PR
6	V-335	TZI-25, Almora	Yellow kernels with flint texture and plant height is medium with good yield	S
7	HUZM-185	Varanasi	Tassel and leaf angle is small. No prolificacy with tall plant height and good grain yield	PS
8	HKI-536	YMNR, Karnal	Early maturity, strong plant, tassel-dense. No prolificacy with good grain yield	PR
9	HKI-287	CML 287, Karnal	Green and narrow leaves, flint, long cob and moderately resistant to TLB	PR
10	HUZM-478	BH-3427, Varanasi	Leaf angle is wide and tassel angle is narrow with flint kernel texture	PR
11	HKI-193	CML193, Karnal	Yellow kernel with flint texture and tall plant height	PR
12	HUZM-53	ISO2 × 1381 WA, Varanasi	Flint kernel texture and late maturity, no prolificacy with high 75% brown husk	R
13	HUZM-121	Varanasi	Medium height with flint-type grain and leaf angle is small with drooping leaf altitude	S
14	HKI-1105	Cargil 633, Karnal	Broad and erect leaves and tall plant height with late maturity	S
15	HUZM-36	PI03011F2-3-5-6-1, Varanasi	Semi-flint kernel texture with tall plant height and high yield with medium size grains	S
16	CM-212	USA/Acc. No.2132 (Alm)-3-2-f-#-13-#, Almora	Early maturity, medium plant and cob height with good yield and highly TLB susceptible	HS
17	CM-145	Pop 31, Almora	Early maturity, tall plant and orange yellow grain, with good yield and straight leaf altitude	HR/R
18	V-336	CML145, P63CDHC181-3-2-1-4#2-BBBB#F-BBBBB#, Almora	Large tassel with light purple glume, flint-dent, orange grains	HR/R
19	V-338	B1045010, Almora	Early maturity, resistance to TLB, days to 50% silking and yield is average	R
20	V-25	Riveirao Preto 8233 (Alm), Almora	Orange grain with straight leaf altitude, tall plant height with good yield	HS/S
21	HKI-162	CML162, Karnal	Small tassel, anther with silk purple and straight leaf altitude with tall plant height	HS/S
22	HUZM-88	HUM242(local) XSuwan1F2, Varanasi	Flint kernel texture with late maturity and tall height with grain yield per plant is low	HS/S
23	HKI-PC-8	LMC 8, Karnal	Drooping broad leaves, resistant to TLB and productive with flint-type kernel texture	R
24	V-348	Derivative of Pop 31, Almora	Anthocyanin colouration is present with straight leaf altitude, tall plant height with good yield	PR
25	V-388	VPKAS, Almora	Anthocyanin colouration is present with straight leaf altitude and better yielder	PR
26	HUZM-47	P502C2-185-3-4-1-3-B-1-B-B, Varanasi	Leaf angle is wide with straight leaf altitude and tall height, good yield with semi-flint-type grains	R
27	HUZM-509	BHU, Varanasi	Leaf angle is small with tassel angle narrow with flint-type grain	PR

(*Continued*)

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Table 1. (*Continued.*)

S. No.	Inbreds	Pedigree/source germplasm	Major characteristics	TLB disease reactions
28	HUZM-81-1	DMR WN-10, Varanasi	Late maturity with flint-type kernels	PR
29	HUZM-356	BHU, Varanasi	Tassel angle is wide with medium height and leaf angle is small	PR
30	HUZM-457	BHU, Varanasi	Medium 75% days to brown husk, leaf angle is small with tassel angle wide	PR
31	HKI-335	Pool 10, Karnal	50% days to silking is low and stay green, purple tassel and silk	PS
32	CM-104	A.Theo21, Amberpet	Late maturity, yellow kernel colour with flint texture	R
33	CM-105	Peru330, Amberpet	Yellow kernel colour with flint texture and having late maturity	R
34	CML-395	90323(B)-1-B-1-B*4/ IITA, Mexico	White kernel colour with dent texture and tall plant height with good yield	S
35	CML-152	S8662Q-1-4-4-5-B-#, Mexico	Leaf angle wide with drooping leaf altitude with tassel anthocyanin colouration present	PS
36	CML-192	G34QMH174-3-1-2-B-B/ Pool 34Q, Mexico	Average plant height and dent-type grains, leaf angle wide with drooping leaf altitude	R
37	CM-126	GCL 33 × Almora local, Almora	Flint kernel texture with early maturity, good yielder, straight leaf altitude	S
38	V-342	VPKAS, Almora	Early maturity with moderately resistance to TLB disease	PS
39	V-346	BIO-45010 OP, F@-2-1-8-5-6, Almora	Days to 50% silking are average, average yield and moderate resistance to TLB	PS
40	V-273	VPKAS, Almora	Moderately resistant to TLB, average grain yield and days to 50% silking are low	PS

HR, highly resistant; R, resistant; PR, partial resistant; PS, partial susceptible; S, susceptible; HS, highly susceptible.

categorized into various clusters and cluster A was solitary, and the inbred line VL171488-2 exhibited a resistant reaction against leaf blight (Keerthana *et al*., 2023). The simple sequence repeat (SSR) markers will allow detection of polymorphisms at the DNA level which will make it easier to distinguish among inbreds. Keerthana *et al*. (2023) reported 26 SSR markers for the assessment of molecular diversity in maize inbred lines for resistance to TLB. Additionally, microsatellite markers have been used by many earlier researchers to analyse the quantitative traits, i.e. TLB resistance and to identify the quantitative trait loci (QTLs) for TLB resistance in maize (Jakhar *et al*., 2022, 2024). QTLs identification helps in marker-assisted selection process for improving genetic traits in crop plants (Singh and Srivastava, 2017). SSR marker system could be used to evaluate TLB-resistant lines because they have a distinct molecular fingerprint compared to susceptible lines. Therefore, the present study was planned to study molecular diversity to identify polymorphic SSR markers for TLB resistance.

Materials and methods

Plant materials and layout

A set of 40 inbred lines including checks (V-336 and CM-145 as resistant check, and CM-212 as susceptible check) were obtained from DMR, New Delhi; VPKAS, Almora and Maize programme, BHU, Varanasi subjected to molecular divergence in maize (Table 1). A study on molecular characterization was carried out in *Kharif* 2015 at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi (located at 25.18°N latitude, 83.03°E longitude and 123.23 m above sea level) using randomized block design with two replications. Each genotype consists of two rows spaced 70 cm apart and 25 cm between plants. Adopting recommended agronomic practices helped to produce a good crop (Mallikarjuna *et al*., 2018). Additionally, at the same time, an investigation was carried out in 2015 to examine TLB reactions with a pure culture of *E. turcicum* in maize under artificial inoculation conditions at two different locations: Nagenahalli, Karnataka, and Varanasi, Uttar Pradesh (Jakhar *et al*., 2021).

DNA extraction and quantification

Total genomic DNA was isolated from 100 mg (21–24 days old) healthy leaf samples collected from individual plants of each inbred lines and stored in a deep freezer at −80°C. The DNA extraction was carried out by using the CTAB procedure described by Saghai-Maroof *et al.* (1984).

SSR marker assay

In the present study, a set of 75 SSR markers (Table 2) were evaluated for 40 maize inbred lines. These SSR markers were received from Applied Biotechnology Centre, CIMMYT, Mexico and the Asian Maize Biotechnology Network (AMBIONET). The primer details for the selected microsatellite markers were retrieved from the maizeGDB (Maize Genetics and Genomics Database) and are publicly accessible (https://www.maizegdb.org/).

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Table 2. List of microsatellite markers used for molecular divergence among TLB-resistant and -susceptible lines of maize

S/N	SSR markers	Repeat type	Bin location	S/N	SSR markers	Repeat type	Bin location
1	bnlg1621	AG(18)	4.06	39	umc1042	GA17	2.07
2	bnlg1019	AG(28)	3.04	40	bnlg1136	AG(14)	6.07–6.08
3	bnlg1917	AG(26)	4.10	41	phi123	AAAG	6.07
4	bnlg1028	AG(12)	10.06	42	bnlg1043	AG(20)	6.00
5	bnlg1094	AG(21)	7.02	43	umc1038	CT(15)	10.07
6	umc1560	(GC)6	2.07	44	umc1051	(GA)7	4.08
7	bnlg1160	AG(13)	3.06	45	bnlg1885	AG(23)	5.07
8	bnlg1350	AG(13)	3.08	46	phi125	AG	8.03
9	phi047	ATC	3.09	47	nc005	CT	4.05
10	bnlg1520	AG(22)	2.09	48	phi112	AG	7.01
11	phi065	CACTT	9.03	49	bnlg2241	AG(26)	3.06
12	bnlg1812	AG(22)	8.05	50	phi101	ACT	5.06
13	bnlg1208	AG(10)	5.04	51	bnlg1138	AG(14)	2.06
14	phi117	ACC	10.00	52	umc1033	(GA)25	9.02
15	bnlg1175	AG(38)	2.04	53	bnlg1588	AG(21)	9.07
16	bnlg1045	AG(23)	2.07	54	bnlg1538	AG(17)	6.01
17	phi053	ATAC	3.05	55	mmc0041	(CA)12	1.08
18	bnlg1338	AG(30)	2.01	56	bnlg1331	AG(16)	1.09
19	bnlg1112	AG(15)	1.01	57	umc1064	(CT)8	1.11
20	mmc0071	(GA)21	3.05–3.10	58	bnlg1836	AG(12)	5.01
21	bnlg1502	AG(17)	1.09–1.10	59	phi021	AG	4.03
22	bnlg1605	AG(18)	3.07	60	umc1067	(GCC)7	4.04
23	phi088	ACT	3.08	61	bnlg2123	AG(31)	1.11
24	mmc0001	(CAA)16	3.09	62	phi016	GGT	9.04
25	bnlg1607	AG(18)	8.06	63	bnlg2238	AG(16)	1.04
26	bnlg1655	AG(21)	10.03	64	phi061	TTCT-GTAT	9.03
27	umc1097	(CA)8	5.00	65	bnlg1169	AG(14)	2.08
28	bnlg1724	AG(31)	9.01	66	umc1084	(CT)23	10.07
29	dupssr6	(CA)6 (A)5 (CA)9	9.02	67	bnlg1209	AG(12)	9.04
30	bnlg1823	AG(18)	8.07	68	umc1076	REALLY LONG CA	1.05
31	dupssr14	(CT)3T(CT)6(CA)16	8.09	69	bnlg1396	AG(15)	2.06
32	umc1027	(CT)6 + EXTRA STUFF	3.06	70	umc1128	TC	1.07
33	bnlg1257	AG(28)	3.09	71	umc1014	(GA)12	6.04
34	bnlg1792	AG(16)	7.02	72	bnlg1367	AG(42)	7.00
35	umc1086	(CT)12	4.08	73	bnlg1217	AG(33)	4.05
36	phi019	ATT	4.11	74	umc1087	(GA)17	3.04
37	dupssr1	(CA)32	5.02	75	bnlg1600	AG(21)	6.00
38	bnlg2328	AG(33)	2.05				

SSR fragment analysis

The polymerase chain reaction (PCR) amplicons of SSR markers were electrophoretically fractionated on a 2.5% agarose gel that was stained with ethidium bromide in 1x TAE buffer. The PCR amplicons were visualized under UV light and photographed using Alpha imager gel documentation system. The 100 bp DNA ladder was used as a molecular weight marker for the analysis of SSR. The distinct PCR amplified products were scored as ‘1’ for the presence and ‘0’ for the absence of DNA bands for each SSR marker genotype combination (Anderson *et al.*, 1993).

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Table 3. Allelic distributions and PIC values of 75 microsatellite markers among 40 TLB-resistant and -susceptible lines

SSR markers	No of alleles	PIC values	SSR markers	No of alleles	PIC values
bnlg1621	3	0.68	umc1042	4	0.73
bnlg1019	2	0.53	bnlg1136	4	0.74
bnlg1917	2	0.50	phi123	5	0.77
bnlg1028	3	0.64	bnlg1043	4	0.74
bnlg1094	3	0.64	umc1038	3	0.62
umc1560	3	0.68	umc1051	4	0.73
bnlg1160	3	0.62	bnlg1885	4	0.74
bnlg1350	3	0.71	phi125	3	0.67
phi047	2	0.56	nc005	4	0.75
bnlg1520	3	0.63	phi112	4	0.74
phi065	3	0.64	bnlg2241	2	0.69
bnlg1812	4	0.78	phi101	2	0.65
bnlg1208	3	0.67	bnlg1138	4	0.75
phi117	2	0.38	umc1033	3	0.64
bnlg1175	2	0.63	bnlg1588	3	0.70
bnlg1045	3	0.65	bnlg1538	3	0.70
phi053	2	0.42	mmc0041	3	0.69
bnlg1338	2	0.46	bnlg1331	4	0.75
bnlg1112	2	0.56	umc1064	3	0.57
mmc0071	3	0.73	bnlg1836	3	0.62
bnlg1502	3	0.67	phi021	2	0.52
bnlg1605	2	0.49	umc1067	2	0.54
phi088	2	0.61	bnlg2123	3	0.66
mmc0001	3	0.67	phi016	2	0.54
bnlg1607	2	0.51	bnlg2238	2	0.47
bnlg1655	2	0.54	phi061	2	0.53
umc1097	4	0.80	bnlg1169	2	0.47
bnlg1724	3	0.71	umc1084	3	0.61
dupssr6	3	0.72	bnlg1209	3	0.58
bnlg1823	2	0.62	umc1076	3	0.66
dupssr14	2	0.61	bnlg1396	4	0.75
umc1027	4	0.72	umc1128	2	0.70
bnlg1257	3	0.64	umc1014	2	0.65
bnlg1792	2	0.65	bnlg1367	2	0.67
umc1086	3	0.68	bnlg1217	3	0.67
phi019	2	0.67	umc1087	2	0.71
dupssr1	4	0.73	bnlg1600	2	0.64
bnlg2328	3	0.69	**Mean**	**2.83**	**0.64**

Data analysis

The informativeness of SSR markers in terms of polymorphism was determined by calculating polymorphic information content (PIC) for each SSR marker loci according to the formula (Anderson *et al.*, 1993):

$$\mathrm{PIC}_i = 1 - \sum p_{ij}^2$$

where p_{ij} is the frequency of the jth microsatellite allele for locus i.

The DNA data of microsatellite primers for maize inbred lines were clustered by using the UPGMA with the module of DARwin

6.0 software. The principal coordinate analysis (PCoA) was estimated to depict the diverse origin of the genotypes by using DARwin 6.0 software (Perrier *et al*., 2003).

Results

Allelic diversity and PIC value

A survey of the molecular profiles generated by the evaluation of amplified products clearly indicated that altogether 212 alleles were detected with an average of 2.83 alleles per markers. Allelic diversity was observed with the number of alleles ranged from two to five. In this study, a direct correlation for the number of alleles and polymorphism information content (PIC) values was observed. For instance, marker phi123 produced high number of alleles (5) with PIC values of 0.77. Similarly other markers such as bnlg1812, umc1097, umc1027, dupssr1, umc1042, bnlg1136, bnlg1043, umc1051, bnlg1885, nc005, phi112, bnlg1138, bnlg1331 and bnlg1396 which also produced higher number of alleles (4) also exhibited high PIC values *viz*., 0.78, 0.80, 0.72, 0.73, 0.73, 0.74, 0.74, 0.73, 0.74, 0.75, 0.74, 0.75, 0.75 and 0.75, respectively (Table 3 and Fig. 1). Furthermore, SSR loci with tetra-nucleotide sequence motifs detected a greater number of alleles than the repeated loci with di-nucleotide, tri-nucleotide and complex sequence motifs.

Genetic relationship among diverse genotypes

The DARwin 6.0 software was used to prepare an UPGMA dendrogram. The genetic distance was used to prepare the UPGMA dendrogram to group 40 maize inbred lines into two major clusters (cluster-I and cluster-II). Cluster-I contained two subclusters, the first subcluster contained 28 genotypes and the second contained eight genotypes whereas cluster-II contained four inbreds. Most of the inbreds except V-25 from cluster-II were highly resistant to TLB disease. This major cluster-II was further classified into two subclusters which contained two genotypes each (Fig. 2).

The PCoA revealed that the 40 maize inbred lines were dispersed into four quadrangles and signifying complex genetic

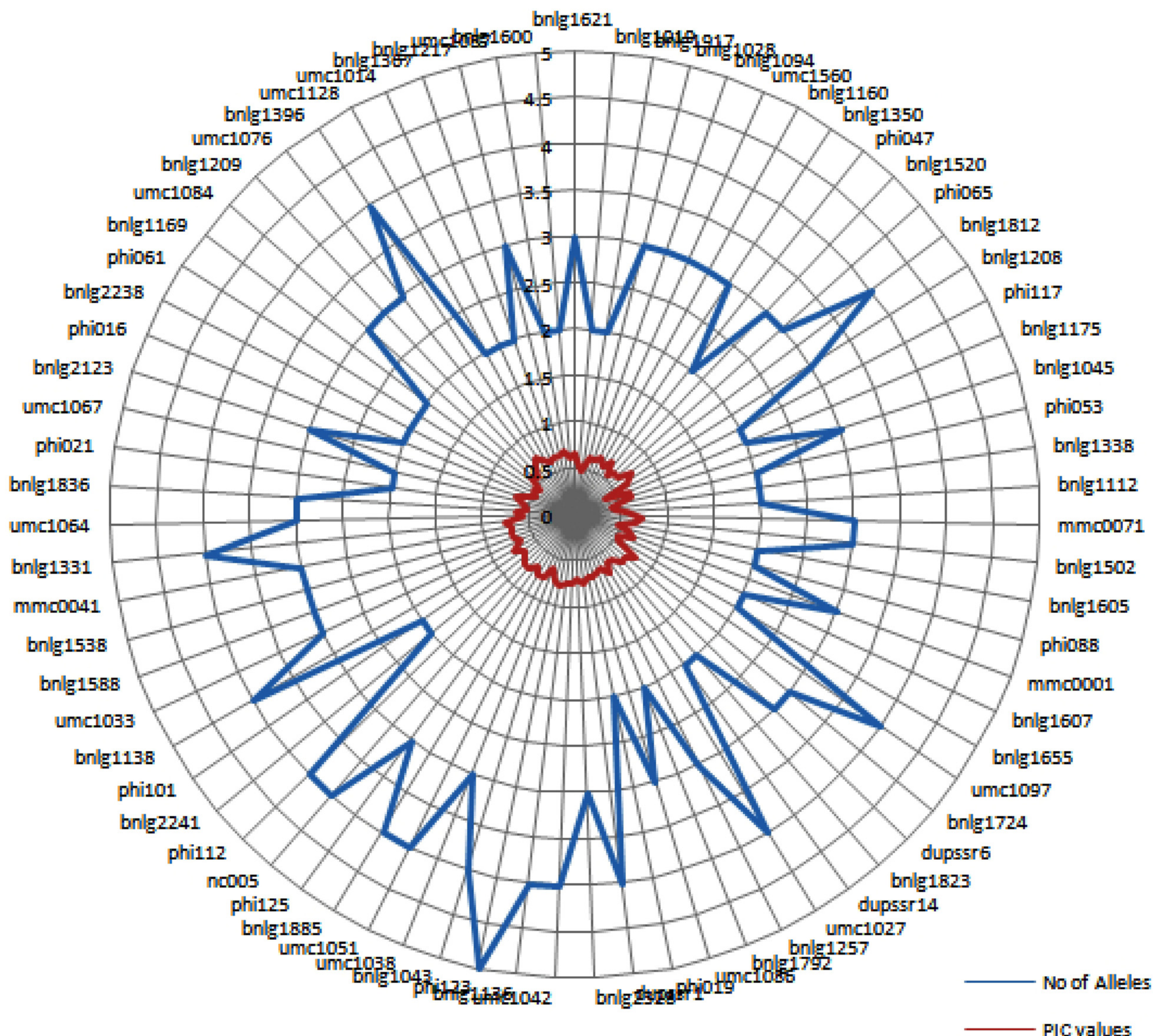

Figure 1. Radar chart for allelic distributions and PIC values of 75 microsatellite markers.

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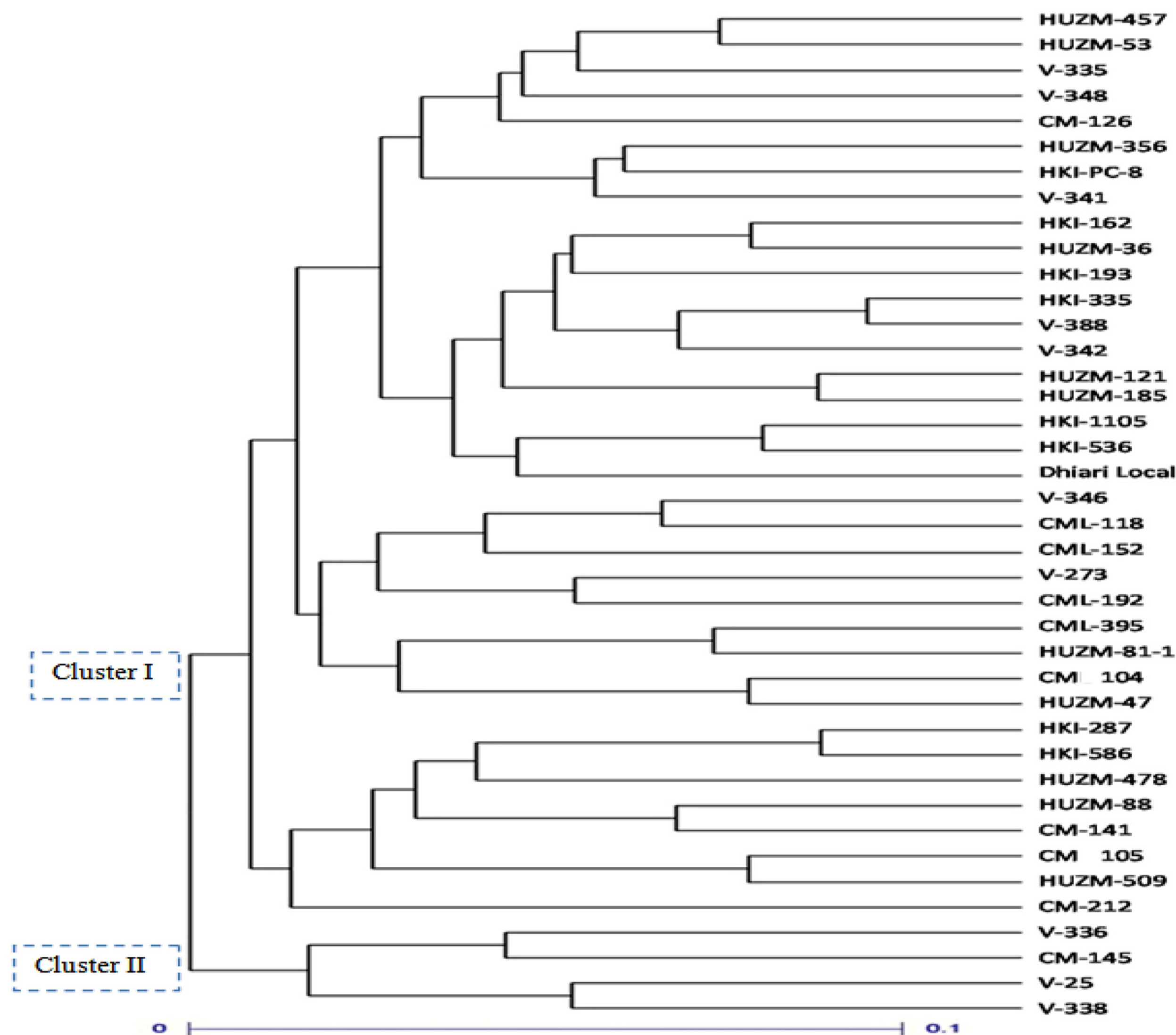

Figure 2. Dendrogram indicating genetic relationship among 40 maize inbred lines generated by using UPGMA method.

relationship among themselves (Fig. 3). The results of the PCoA supported the UPGMA dendrogram's clustering pattern and nearly coincided with the findings of the cluster analysis.

Discussion

The UPGMA dendrogram grouped 40 maize inbreds into two major clusters (cluster-I and cluster-II). Cluster-I contained two subclusters, the first subcluster contained 28 genotypes and the second contained eight genotypes whereas cluster II contained 4 inbreds. This major cluster II was further classified into two subclusters which contained two genotypes each. Therefore, TLB-resistant maize inbreds were unambiguously differentiated from susceptible and partially resistant maize inbred lines.

The 75 microsatellite marker loci differed in their ability to determine the variability among 40 TLB-resistant and -susceptible lines of maize based on their genetic polymorphism. The value of PIC of a marker reflects primer specific gene diversity, and frequency among the inbreds. The higher value of PIC of a SSR marker indicated a higher number of alleles among inbreds (Kumari *et al*., 2018). A sum total of 212 alleles were found at 75 SSR loci with a mean of 2.83 alleles per SSR locus. The number of alleles ranged from two to five with an average of 2.83 alleles per markers. The average number of alleles in the present study is comparable with earlier genetic diversity analysis (Jaya Kumar, 2010), who reported 2–8 alleles per marker. Similar studies have also been conducted in maize (Yuan *et al*., 2000; Prasanna and Hoisington, 2003; Choukan *et al*., 2006).

In this study, a positive correlation for the number of alleles and PIC values was found. For instance, marker phi123 produced high number of alleles (5) with PIC values of 0.77. Furthermore, SSR loci with tetra-nucleotide sequence motifs found a maximum number of alleles than the repeated loci with di-nucleotide, tri-nucleotide and complex sequence motifs in the present investigation. Generally, the di-nucleotide repeat motifs were found to be more polymorphic than those with tri-nucleotide, tetra-nucleotide and complex repeat motifs (Lapitan *et al*., 2007; Kumar *et al*., 2018; Kumari *et al*., 2018). The importance of SSR markers for the assessment of genetic diversity has been reported by earlier workers (Li *et al*., 2002; Xia *et al*., 2004; Yu *et al*., 2007; Reid *et al*., 2011; Haasbroek *et al*., 2014) by using maize genotypes.

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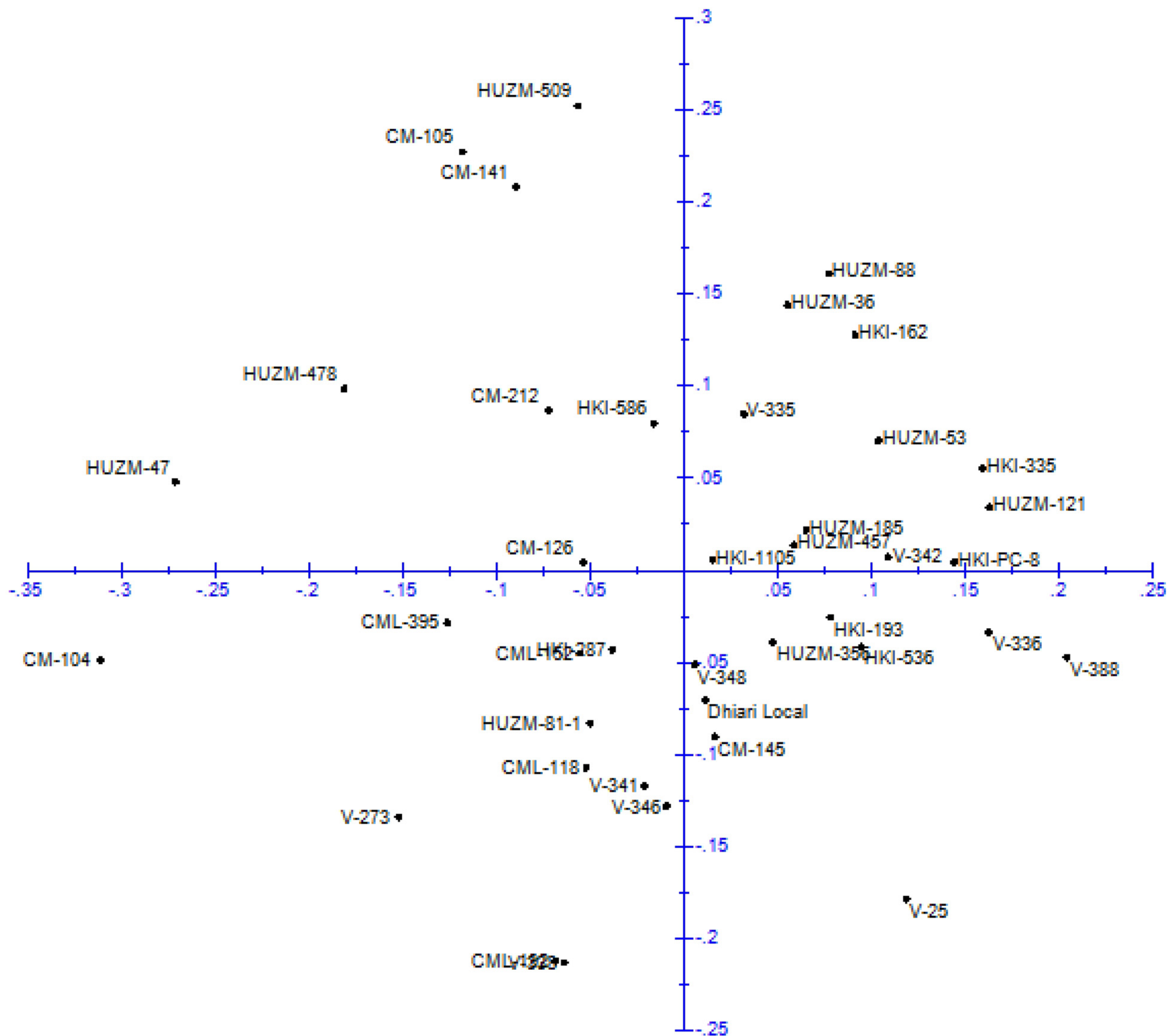

Figure 3. Principal coordinate analysis (PCoA) among 40 maize inbreds using 75 SSR markers.

In the present investigation, 75 SSR markers revealed sufficiently high sensitivity for detecting DNA polymorphism among the 40 maize inbreds. The dendrogram revealed the genotypic relationship among each other to explore their utility for further studies.

The PCoA showed that the distributions of 40 maize inbreds in the four quadrangles were highly dispersed, signifying complex genetic relationship among themselves. In the present study, the results of PCoA also illustrated the diverse nature of the maize inbreds and agreed well with their cluster analysis pattern. The diverse nature of the maize inbreds has already been proven to be a good source for the development of superior heterotic hybrids by Solomon *et al.* (2012), Babic *et al.* (2014) and Mehta *et al.* (2017). Further investigations are required to evaluate whether the discrimination between resistant and susceptible genotypes is consistent for other isolates of *E. turcicum* and during natural infection in different environmental conditions in multiple years.

Conclusions

In the present investigation, 75 microsatellite markers were used for the assessment of molecular divergence among 40 TLB-resistant and -susceptible lines of maize. Among these SSR markers, Phi123 produced a large number of alleles (5) with PIC values of 0.77. Similarly, other markers that produced a greater number of alleles, such as bnlg1812, umc1097, umc1027, dupssr1, umc1042, bnlg1136, bnlg1043, umc1051, bnlg1885, nc005, phi112, bnlg1138, bnlg1331 and bnlg1396, also showed a high PIC value. Furthermore, these markers observed a significant amount of variation in the resistant genotypes indicating their usefulness. In this study, most of the inbred lines from cluster-II were highly resistant

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to TLB disease. These TLB-resistant lines can be used as the divergent parents to develop TLB-resistant hybrids. Allelic diversity and PIC values in this investigation showed that the markers used were effective in determining the polymorphism level found in the studied inbred lines. Furthermore, the notable level of divergence detected by SSR markers can be used in crossing programmes to develop TLB-resistant hybrids. The findings of this study can also be used to design efficient breeding programmes for resistance to TLB.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262124000315

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