CHAPTER VII

PLANT BIOTECHNOLOGY

PLANT BIOTECHNOLOGY

PMMB 1: TRAIT ASSOCIATION STUDIES AND VALIDATION OF MARKERS IN PEARL MILLET

Experiment 1: Trait association studies and validation of drought tolerance markers in drought/heat tolerant pearl millet genotypes suitable for A_1 zone.

Plant Material

A total of 24 pearl millet drought/heat tolerant lines suitable for A_1 zone were used for morphological and molecular characterization under this experiment (Table 1.1). Development of high yielding, dual purpose disease resistant cultivars for low rainfall areas i.e. A_1 zone is of utmost priority for increasing pearl millet productivity at national level and develop some hybrids for this specific zone. Keeping this in view, this experiment was useful for screening and developing drought/heat tolerant hybrids for A_1 zone.

Genomic DNA isolation and quantification

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of 24 pearl millet lines using the CTAB method (Murray and Thompson, 1980) with slight modifications and it was also observed that good quality DNA was extracted even without using liquid nitrogen for grinding of hard leaf tissues like pearl millet which can reduce cost of isolation.

Table 1.1 List of pearl millet lines used for molecular characterization

S. No.	Name of line	Source
1	MIR 525-2 (BM/K-18/322)	ICAR-AICRP on Pearl millet, Jodhpur
2	MIR 1106 (BM/K-18/368)	ICAR-AICRP on Pearl millet, Jodhpur
3	MIR 1262(BM/K-18/404)	ICAR-AICRP on Pearl millet, Jodhpur
4	MIR 1356 (BM/K-18/436)	ICAR-AICRP on Pearl millet, Jodhpur
5	MIR 1408 (BM/K-18/478)	ICAR-AICRP on Pearl millet, Jodhpur
6	MIR 1604 (BM/K-18/496)	ICAR-AICRP on Pearl millet, Jodhpur
7	MIR 1706 (BM/K-18/506)	ICAR-AICRP on Pearl millet, Jodhpur
8	MIR 1803 (BM/K-18/544)	ICAR-AICRP on Pearl millet, Jodhpur
9	RIB 128-134/S/19	RARI, Durgapura
10	RIB 156-162/S/19	RARI, Durgapura
11	RIB 163-169/S/19	RARI, Durgapura
12	RIB 170-176/S/19	RARI, Durgapura
13	RIB 177-183	RARI, Durgapura
14	RIB 184-190/S/19	RARI, Durgapura
15	H77/833/2-202	HAU, Hisar
16	G 73-107	HAU, Hisar
17	H 77/833-2	HAU, Hisar
18	H 90/4-5	HAU, Hisar
19	HBL 11	HAU, Hisar
20	BIB-437	SKRAU, Bikaner
21	BIB-445	SKRAU, Bikaner
22	ICMB 99777	ICRISAT, Patancheru
23	ICMB 95222	ICRISAT, Patancheru
24	ICMB 97444	ICRISAT, Patancheru

Molecular characterization and SSR analysis

For PCR reaction, DNA was diluted accordingly to make available final concentration of 10 ng/µl and amplification reactions were carried out in a volume of 10 µl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit Taq NA polymerase (GeNei, India) and 10 ng of template DNA. Amplifications were carried out in a 96-well thermal cycler (Agilent Technologies). Thermal cycler was programmed to 1 cycle of 5 min at 94°C for initial strand separation. This was followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 58°C for annealing and 1 min at 72°C for primer extension. Finally, 1 cycle of 10 mins at 72°C was used for final extension, followed by hold at 4°C. A total of 15 SSR primers specifically reported for drought were used for PCR amplification and molecular characterization among 24 pearl millet lines. All the 15 SSRs amplified products of varying sizes ranging between 100-600 bp (Fig. 1.1). A total of 35 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.73 alleles. Polymorphic Information Content (PIC) varied from 0.43 to 0.84 with an average of 0.67 PIC value. Four SSR markers (PSMP2059, PSMP2077, PSMP2078 and PSMP2066) linked to drought-tolerant QTLs on LG2 and LG5 identified by Yadav et al. (2004) were also validated here among these genotypes.

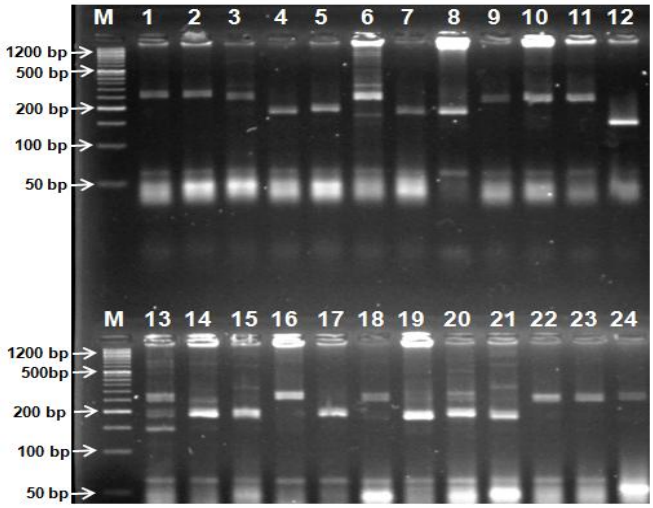

Fig. 1.1 Agarose gel showing amplification profiles of pearl millet lines using the primer PSMP 2066. Lane M-50 bp ladder, Lane 1-24 pearl millet lines

Morphological parameters

A total of 24 pearl millet drought/heat tolerant lines suitable for A_1 zone were evaluated for morpho-physiological conditions and different parameters were recorded. Relative Water Content (RWC) at 45 DAS for different genotypes ranged from 66.9 (MIR 525-2) to 83.41 (RIB-163-169). Chlorophyll Content at 45 DAS for different genotypes ranged from 1.01 (RIB-163-169) to 1.95 (BIB 437). Membrane Stability index at 45 DAS varied from 67.92 (MIR 1262) to 86.63 (H77/833-2). Number of tillers/plant for different genotypes ranged from 3.00 (ICMB 97777) to 6.07 (ICMB 95222) while no. of productive tillers/plant ranged from 1.53 (RIB-156-162) to 4.23 (H 90/4-5). Flag leaf area varied from 51.45 (MIR 1262) to 90.87 cm^2 (ICMB 95222). Plant height for different genotypes ranged from 96.44 (ICMB 97444) to 154.51 cm

(ICMB 95222). The spike length varied from 14.93 (HBL 11) to 20.07 cm (ICMB 97777) whereas spike thickness varied from 1.38 (H77/833-2-202) to 2.76 cm (ICMB 97777). Days to 50% flowering for different genotypes ranged from 38.67 (MIR 525-2, MIR 1106) to 52.00 (ICMB 97444). Test weight varied from 5.34 (MIR 1408) to 10.47 g (ICMB 97444) while grain yield/plant varied from 15.93 (MIR 1803) to 32.67 g (ICMB 97777).

Experiment 2: Validation of markers and study trait association for diseases in pearl millet.

Plant Material

A total of 38 entries of downy mildew nursery trials and 57 entries of blast nursery trials were used for molecular characterization using markers identified for downy mildew and blast, respectively under this experiment (Table 1.2, 1.3). Downy mildew and blast are two major diseases affecting pearl millet. Hence, screening of pearl millet lines resistant to these diseases will be useful for developing disease resistant pearl millet hybrids/varieties.

Genomic DNA isolation and quantification

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of 38 entries of downy mildew nursery trials and 57 entries of blast nursery trials as described in PMMB 1 and quantified on agarose gel.

Table 1.2 List of entries of downy mildew nursery trials

S. No.	Entry Code	S. No.	Entry Code
1	ICPMDML-1	20	ICPMDML-20
2	ICPMDML-2	21	ICPMDML-21
3	ICPMDML-3	22	ICPMDML-22
4	ICPMDML-4	23	ICPMDML-23
5	ICPMDML-5	24	ICPMDML-26
6	ICPMDML-6	25	ICPMDML-27
7	ICPMDML-7	26	ICPMDML-28
8	ICPMDML-8	27	ICPMDML-29
9	ICPMDML-9	28	ICPMDML-30
10	ICPMDML-10	29	ICPMDML-31
11	ICPMDML-11	30	ICPMDML-32
12	ICPMDML-12	31	ICPMDML-33
13	ICPMDML-13	32	ICPMDML-34
14	ICPMDML-14	33	ICPMDML-35
15	ICPMDML-15	34	ICPMDML-36
16	ICPMDML-16	35	ICPMDML-37
17	ICPMDML-17	36	ICPMDML-38
18	ICPMDML-18	37	ICPMDML 39
19	ICPMDML-19	38	ICPMDML-40

Table 1.3 List of entries of blast nursery trials

S. No.	Entry Code	S. No.	Entry Code
1	ICPMBL-1	30	ICPMBL-30
2	ICPMBL-2	31	ICPMBL-31
3	ICPMBL-3	32	ICPMBL-32
4	ICPMBL-4	33	ICPMBL-33
5	ICPMBL-5	34	ICPMBL-34
6	ICPMBL-6	35	ICPMBL-35
7	ICPMBL-7	36	ICPMBL-36
8	ICPMBL-8	37	ICPMBL-37
9	ICPMBL-9	38	ICPMBL-38
10	ICPMBL10	39	ICPMBL-39
11	ICPMBL-11	40	ICPMBL-40
12	ICPMBL-12	41	ICPMBL-41
13	ICPMBL-13	42	ICPMBL-42
14	ICPMBL-14	43	ICPMBL-43
15	ICPMBL-15	44	ICPMBL-44
16	ICPMBL-16	45	ICPMBL-45
17	ICPMBL-17	46	ICPMBL-46
18	ICPMBL-18	47	ICPMBL-47
19	ICPMBL-19	48	ICPMBL-48
20	ICPMBL-20	49	ICPMBL-49
21	ICPMBL-21	50	ICPMBL-50
22	ICPMBL-22	51	ICPMBL-51
23	ICPMBL-23	52	ICPMBL-52
24	ICPMBL-24	53	ICPMBL-53
25	ICPMBL-25	54	ICPMBL-54
26	ICPMBL-26	55	ICPMBL-55
27	ICPMBL-27	56	ICPMBL-56
28	ICPMBL-28	57	ICPMBL-57
29	ICPMBL-29		

Molecular characterization and SSR analysis

For PCR reaction, DNA was diluted accordingly to make available final concentration of 10 ng/µl and amplification reactions were carried out in a volume of 10 µl as described in PMMB1. A total of 56 SSR primers were used for PCR amplification with 38 entries of downy mildew nursery trials. Out of 56 SSRs, 48 primers amplified products of varying sizes ranging from 90 to 600 bp and 30 (53.6%) were polymorphic and 18 (32.1 %) were monomorphic (Table 1.4 and Fig. 1.2). A total of 76 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.53 alleles**.** Polymorphic Information Content (PIC) varied from 0.39 to 0.79 with an average of 0.54 PIC value.

Table 1.4 Results of SSR primers used for PCR

Markers	Markers for DM	Markers for blast
Number of SSR markers used	56	58
Number of amplified markers	48	47
Number of non- amplified markers	08	11
Number of polymorphic markers	30	34
Number of monomorphic markers	18	13

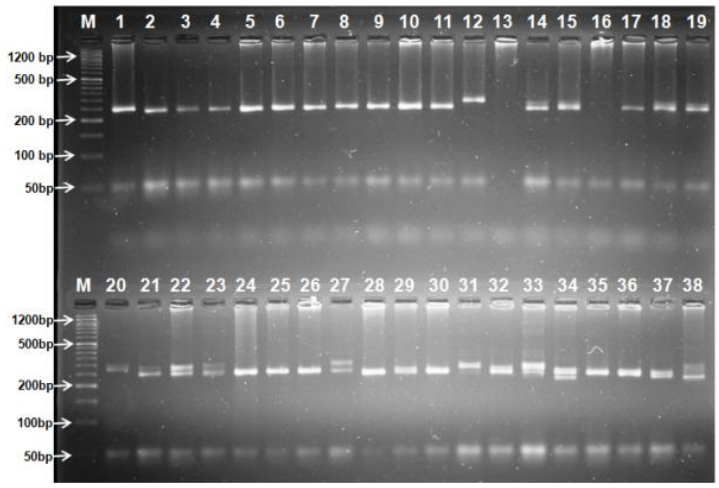

Fig. 1.2 Agarose gel showing amplification profiles of entries of downy mildew nursery trials using the primer PSMP 2008. Lane M-50 bp ladder, Lane 1-38 entries of pearl millet downy mildew nursery trials

A total of 58 SSR primers were used for PCR amplification with 57 entries of blast nursery trials. Out of 58 SSRs, 47 primers amplified products of varying sizes ranging from 120 to 570 bp and 34 (58.6 %) were polymorphic and 13 (22.4%) were monomorphic (Table 1.4 and Fig. 1.3). A total of 86 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.52 alleles**.** Polymorphic Information Content (PIC) varied from 0.32 to 0.79 with an average of 0.52 PIC value.

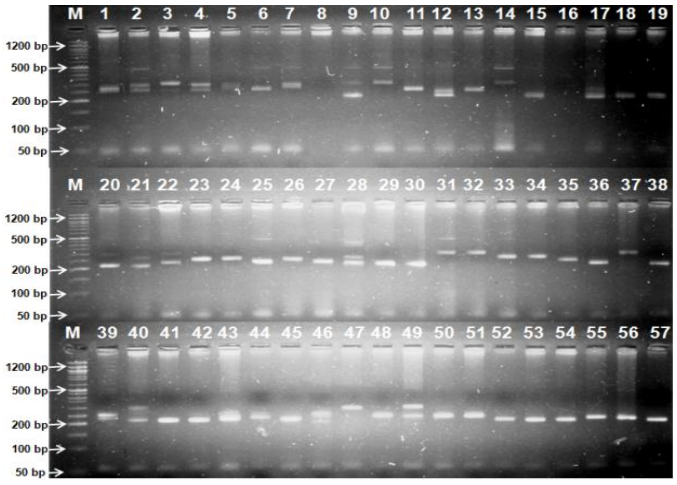

Fig. 1.3 Agarose gel showing amplification profiles of entries of blast nursery trials using the primer PSMP2084. Lane M-100 bp ladder, Lane 1-57 entries of pearl millet blast nursery trials

Morphological parameters

A total of 38 entries of downy mildew nursery trials and 57 entries of blast nursery trials were screened in field conditions and data were recorded by counting no. of diseased plants at 60 DAS. Analysis of the downy mildew disease incidence showed one or more typical downy mildew symptoms and revealed significant differences among the different entries. The disease incidence ranged between 3.33% (ICPMDML-11) to 24.59% (ICPMDML-4) among the entries screened at Mandor. 13 entries with 0-5% infection were found to be resistant, 19 entries with >5 to 10% disease incidence were found to be moderately resistant and 7 entries with >10-25 % disease incidence were categorized to be moderately susceptible.

A total of 57 entries of blast nursery trials were screened in field conditions and data were recorded at 60 DAS. Analysis of the blast disease incidence showed one or more blast disease symptoms and revealed significant differences among the different entries. The disease incidence ranged between 1 (ICPMBL-14) to 6.5 (ICPMBL-54) among the entries screened at Mandor. 34 entries with score 1-3.5 were found to be resistant, 22 entries with score 4-5.5 were found to be moderately resistant and 1 entry (ICPMBL-54) with 6.5 score was found to be susceptible.

Experiment 3: Validation and association studies for high Fe/Zn among pearl millet lines rich in Fe and Zn content

Plant Material

A total of 38 pearl millet lines rich in Fe/Zn were used for molecular characterization and screening and validation of already reported high Fe/Zn markers under this experiment (Table 1.5). Pearl millet is rich in Fe & Zn content and ICAR-AICRP on Pearl Millet has already included minimum standard for micronutrient (Fe = 42 ppm; Zn = 32 ppm) in the promotion criteria. Hence, screening of pearl millet lines rich in Fe and Zn content using molecular markers and their validation will be helpful for developing high Fe/Zn pearl millet hybrids.

Genomic DNA isolation and quantification

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of 38 genotypes as described in PMMB1 and quantified on agarose gel.

Table 1.5 List of genotypes used for molecular characterization

S. No.	Name of pearl millet line	Organization	Fe (ppm)	Zn (ppm)
1.	PPMI 1322	IARI, New Delhi	35	32
2.	PPMI 1323	IARI, New Delhi	53	39
3.	PPMI 1324	IARI, New Delhi	71	48
4.	PPMI 1325	IARI, New Delhi	57	45
5.	PPMI 1326	IARI, New Delhi	54	44
6.	HSR 24-1	CCS HAU, Hisar	30	25
7.	HSR 24-2	CCS HAU, Hisar	40	40
8.	HSR 24-3	CCS HAU, Hisar	41	30
9.	HSR 24-4	CCS HAU, Hisar	53	42
10.	150 SB 24	JAU, Jamnagar	62	44

S. No.	Name of pearl millet line	Organization	Fe (ppm)	Zn (ppm)
11.	159 SB 24	JAU, Jamnagar	33	33
12.	171 SB 24	JAU, Jamnagar	40	34
13.	217 SB 24	JAU, Jamnagar	41	31
14.	229 SB 24	JAU, Jamnagar	50	33
15.	JMSB 20213	JAU, Jamnagar	49	37
16.	JMSB 20227	JAU, Jamnagar	75	38
17.	JMSB 20229	JAU, Jamnagar	43	34
18.	DHL CRP 01	MPKV, Dhule	59	38
19.	DHL CRP 02	MPKV, Dhule	52	42
20.	DHL CRP 03	MPKV, Dhule	59	41
21.	DHL CRP 04	MPKV, Dhule	60	42
22.	DHL CRP 05	MPKV, Dhule	51	33
23.	DHL CRP 06	MPKV, Dhule	51	41
24.	RIB 15178	RARI, Durgapura	53	36
25.	RIB 15179	RARI, Durgapura	57	39
26.	RIB 15180	RARI, Durgapura	44	35
27.	RIB 15181	RARI, Durgapura	38	45
28.	RIB 15182	RARI, Durgapura	27	27
29.	RIB 15183	RARI, Durgapura	42	33
30.	RIB 15184	RARI, Durgapura	57	36
31.	RIB 15185	RARI, Durgapura	58	45
32.	RIB 15186	RARI, Durgapura	54	36
33.	RIB 15187	RARI, Durgapura	45	36
34.	MIR 1117	PC Unit, Jodhpur	53	39
35.	MIR 1406	PC Unit, Jodhpur	60	56
36.	MIR 1716	PC Unit, Jodhpur	37	35
37.	MIR 1806	PC Unit, Jodhpur	52	40
38.	MIR 1907	PC Unit, Jodhpur	44	42

Molecular characterization and SSR analysis

A total of 112 SSR primers were used for PCR amplification and validation among 38 pearl millet genotypes. Out of 112 SSRs, 91 primers amplified products of varying sizes ranging from 110 to 750 bp and 71 (63.4%) were polymorphic and 20 (17.9 %) were monomorphic (Table 1.6 and Fig. 1.4). A total of 194 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.73 alleles**.** Polymorphic Information Content (PIC) varied from 0.35 to 0.78 with an average of 0.59 PIC value.

Table 1.6 Results of SSR primers used for PCR

Markers	No. of markers
Number of markers used	112
Number of amplified markers	91
Number of non- amplified markers	21
Number of polymorphic markers	71
Number of monomorphic markers	20

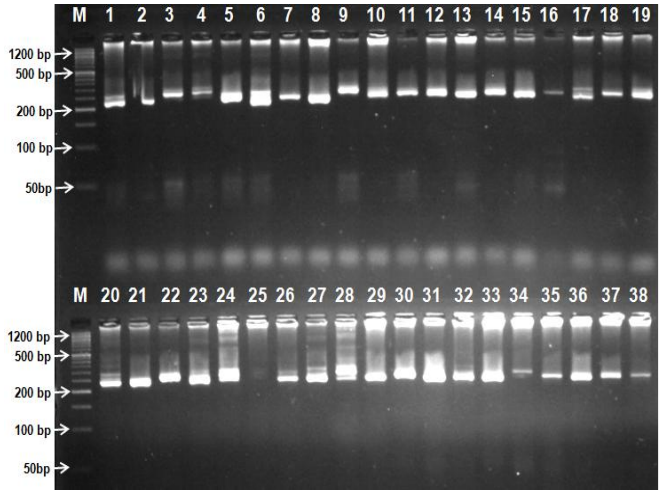

Fig. 1.4 Agarose gel showing amplification profiles of pearl millet lines using the primer IPES 0203. Lane M-50 bp ladder, Lane 1-38 pearl millet lines

Morphological characterization

Observations recorded for days to 50% flowering, effective tillers per plant, plant height, head diameter, head length etc. Days to 50% flowering ranged from 48 (PPMI 1323) to 65 (RIB 15181 and RIB 15186). Plant height ranged from 85 cm (JMSB 20227 and DHL CRP 01) to 163 cm (JMSB 20229). Effective tillers per plant was 1.5 (PPMI 1322, HSR 24-2, 217 SB 24, RIB 15178, RIB 15179 and RIB 15183) to 4 cm (MIR 1716). Head length ranged from 11 cm (HSR 24-4, DHL CRP 01) to 35 cm (JMSB 20229). Head girth ranged from 1.8 cm (MIR 1117) to 4 cm (HSR 24-2). The seed set (%) under open pollination condition ranged from 33 (RIB 15183) to 88 (MIR 1907). Agronomic score ranged from 1.8 (HSR 24-4) to 4.5 (MIR 1907). Iron content ranged from 27 (RIB 15182) to 75 ppm (JMSB 20227) while zinc content ranged from 25 (HSR 24-1) to 56 ppm (MIR 1406) (Table 1.7).

Table 1.7 Performance of different pearl millet genotypes under field conditions

S. No.	Pearl millet line	Days to 50% flowering	Plant Height (cm)	Effective Tiller/plant	Head Length (cm)	Head Dia Meter (cm)	Open Pollination Seed set %	Agronomic Score (1-5), 1=Poor, 5=Excellent	Fe (ppm)	Zn (ppm)
1	PPMI 1322	61	118	1.5	22	1.9	73	3.3	35	32
2	PPMI 1323	48	148	2.5	21	2.3	73	3.3	53	39
3	PPMI 1324	61	110	1.8	15	2.3	55	2.3	71	48
4	PPMI 1325	56	150	2.5	21	3.2	65	3.5	57	45
5	PPMI 1326	62	135	1.8	21	2.4	55	2.5	54	44
6	HSR 24-1	52	122	3.0	13	1.9	73	4.3	30	25
7	HSR 24-2	61	105	1.5	16	4.0	76	3.0	40	40
8	HSR 24-3	59	105	2.3	14	3.2	68	2.3	41	30
9	HSR 24-4	58	105	2.0	11	2.6	48	1.8	53	42
10	150 SB 24	55	145	3.5	17	3.0	60	3.3	62	44
11	159 SB 24	55	123	2.0	18	2.1	48	2.3	33	33
12	171 SB 24	52	135	2.0	21	2.3	63	2.3	40	34
13	217 SB 24	61	108	1.5	20	2.4	47	2.3	41	31
14	229 SB 24	55	148	2.3	27	2.9	53	2.0	50	33

S. No.	Pearl millet line	Days to 50% flowering	Plant Height (cm)	Effective Tiller/plant	Head Length (cm)	Head Dia Meter (cm)	Open Pollination Seed set %	Agronomic Score (1-5), 1=Poor, 5=Excellent	Fe (ppm)	Zn (ppm)
15	JMSB 20213	60	117	2.8	21	2.9	60	2.3	49	37
16	JMSB 20227	64	85	3.0	16	3.8	80	3.3	75	38
17	JMSB 20229	64	163	2.8	35	3.2	70	2.3	43	34
18	DHL CRP 01	55	85	2.0	11	2.7	40	2.0	59	38
19	DHL CRP 02	61	138	2.5	17	3.1	44	2.5	52	42
20	DHL CRP 03	63	143	2.5	20	3.4	63	4.3	59	41
21	DHL CRP 04	62	148	2.3	21	3.5	50	3.3	60	42
22	DHL CRP 05	63	153	2.0	23	3.6	55	2.3	51	33
23	DHL CRP 06	60	153	3.0	23	3.5	55	2.3	51	41
24	RIB 15178	58	105	1.5	15	2.6	50	2.3	53	36
25	RIB 15179	64	105	1.5	16	3.0	80	2.3	57	39
26	RIB 15180	57	103	2.3	17	3.0	50	2.3	44	35
27	RIB 15181	65	133	2.5	19	3.1	48	2.3	38	45
28	RIB 15182	51	135	2.5	19	3.2	73	2.3	27	27
29	RIB 15183	64	128	1.5	19	2.4	33	2.3	42	33
30	RIB 15184	59	155	2.5	24	2.9	75	3.3	57	36
31	RIB 15185	64	113	1.8	19	3.8	85	3.8	58	45
32	RIB 15186	65	100	2.0	18	3.9	75	3.0	54	36
33	RIB 15187	57	138	2.3	20	3.6	65	2.8	45	36
34	MIR 1117	64	125	3.0	18	1.8	85	3.3	53	39
35	MIR 1406	55	103	2.3	12	3.3	70	3.5	60	56
36	MIR 1716	54	130	4.0	19	1.9	83	4.0	37	35
37	MIR 1806	53	120	3.8	22	2.0	80	4.0	52	40
38	MIR 1907	53	105	2.8	12	3.4	88	4.5	44	42

PMMB 2: MOLECULAR CHARACTERIZATION OF GERMPLASM OF PEARL MILLET

Experiment 1: DNA profiling of identified/advanced hybrid entries of third year testing

Plant Material

The four advanced hybrid entries (MH 2672, MH 2673, MH 2675 and MH 2678) of third year testing from AHPT I (E) A to AHPT II (E) trial of A_1 zone along with two checks (MPMH21, AHB1200) and three advanced hybrid entries (MH 2709, MH 2712 and MH 2717) of third year testing from AHT I (L) A to AHT II (L) trial along with two checks (KBH108, 86M86) were used for DNA profiling under this experiment. The entries promoted for third year testing will be later identified for release and hence need DNA profile for submission of proposal. Thus, this experiment is useful for authenticity of identification proposal and can meet the basic requirements of proposal submission.

Genomic DNA isolation and quantification

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of seven advanced hybrid entries and four checks as described in PMMB1 and quantified on agarose gel.

DNA profiling using SSRs

For PCR reaction, DNA was diluted accordingly to make available final concentration of 10 ng/µl and amplification reactions were carried out in a volume of 10 µl as described in PMMB 1. A total of 36 SSR primers were used for PCR amplification and DNA profiling of advanced hybrid entries of pearl millet. Among the advanced hybrid entries (MH 2672, MH 2673, MH 2675 and MH 2678) of third year testing from AHPT I (E) A to AHPT II (E) trial, analysis of MH 2672 using 36 primers displayed a unique profile for this genotype (Fig. 2.1). Out of the 36 primers used, 21 SSR primers (58.3%) were found polymorphic between the three genotypes. Similarly, analysis of MH2673 using the same 36 primers displayed a unique profile for this genotype (Fig. 2.2). Out of the 36 primers used, 19 SSR primers (52.7%) were found polymorphic between the three genotypes. Analysis of MH2675 using the same 36 primers displayed a unique profile for this genotype (Fig. 2.3). Out of the 36 primers used, 16 SSR primers (44.4%) were found polymorphic between the three genotypes. Analysis of MH2678 using the same 36 primers displayed a unique profile for this genotype (Fig. 2.4). Out of the 36 primers used, 19 SSR primers (52.7%) were found polymorphic between the three genotypes.

Among the three advanced hybrid entries (MH 2709, MH 2712 and MH 2717) of third year testing from AHT I (L) A to AHT II (L) trial, analysis of MH 2709 using 36 primers displayed a unique profile for this genotype (Fig. 2.5). Out of the 36 primers used, 17 SSR primers (47.2 %) were found polymorphic between the three genotypes. Similarly, analysis of MH 2712 using the same 36 primers displayed a unique profile for this genotype (Fig. 2.6). Out of the 36 primers used, 18 SSR primers (50.0 %) were found polymorphic between the three genotypes. Analysis of MH2717 using the same 36 primers displayed a unique profile for this genotype (Fig. 2.7). Out of the 36 primers used, 19 SSR primers (52.7 %) were found polymorphic between the three genotypes.

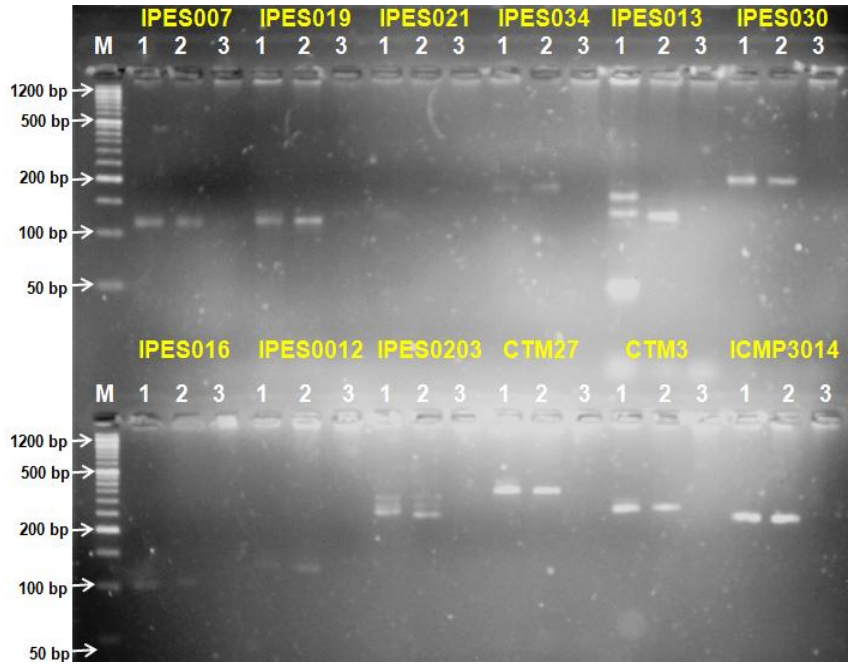

Fig 2.1 Gel photograph showing SSR allelic profile of MH2672 with 36 SSRs. M-50 bp DNA ladder; Lane 1- MPMH21 (Check), 2- AHB1200 (Check), 3- MH2672

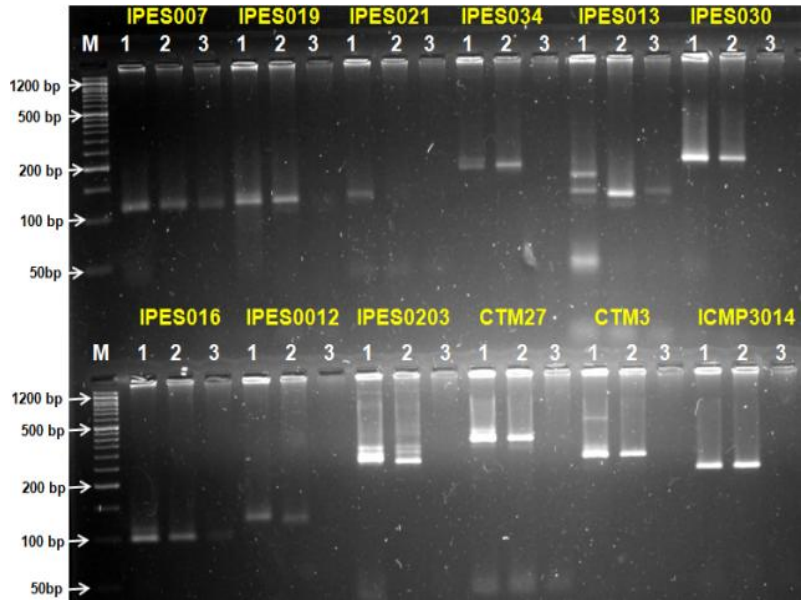

Fig 2.2 Gel photograph showing SSR allelic profile of MH2673 with 36 SSRs. M-50 bp DNA ladder; Lane 1- MPMH21 (Check), 2- AHB1200 (Check), 3- MH2673

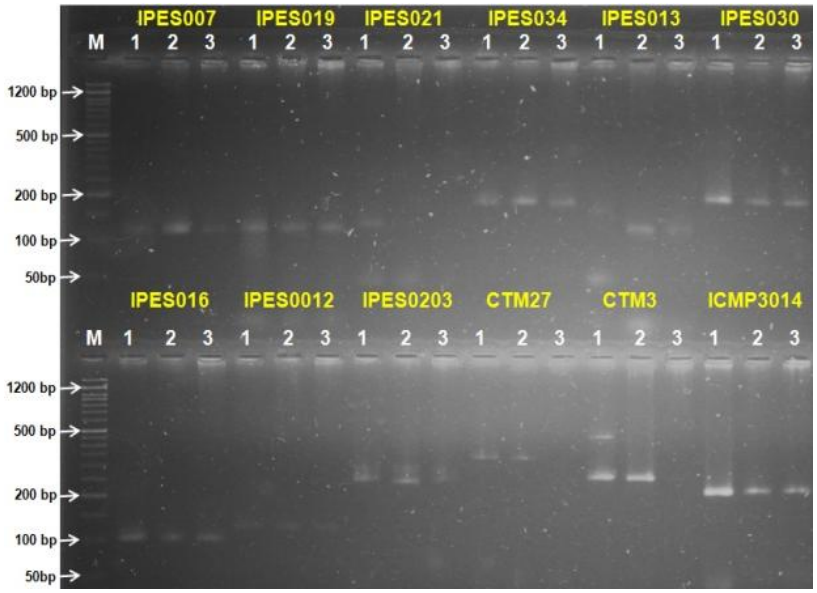

Fig 2.3 Gel photograph showing SSR allelic profile of MH2675 with 36 SSRs. M-50 bp DNA ladder; Lane 1- MPMH21 (Check), 2- AHB1200 (Check), 3- MH2675

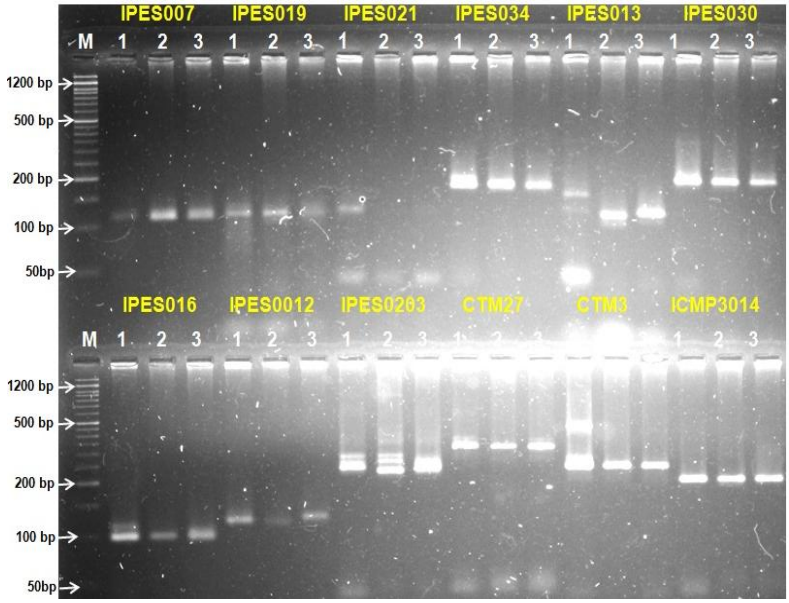

Fig 2.4 Gel photograph showing SSR allelic profile of MH2678 with 36 SSRs. M-50 bp DNA ladder; Lane 1- MPMH21 (Check), 2- AHB1200 (Check), 3- MH2678

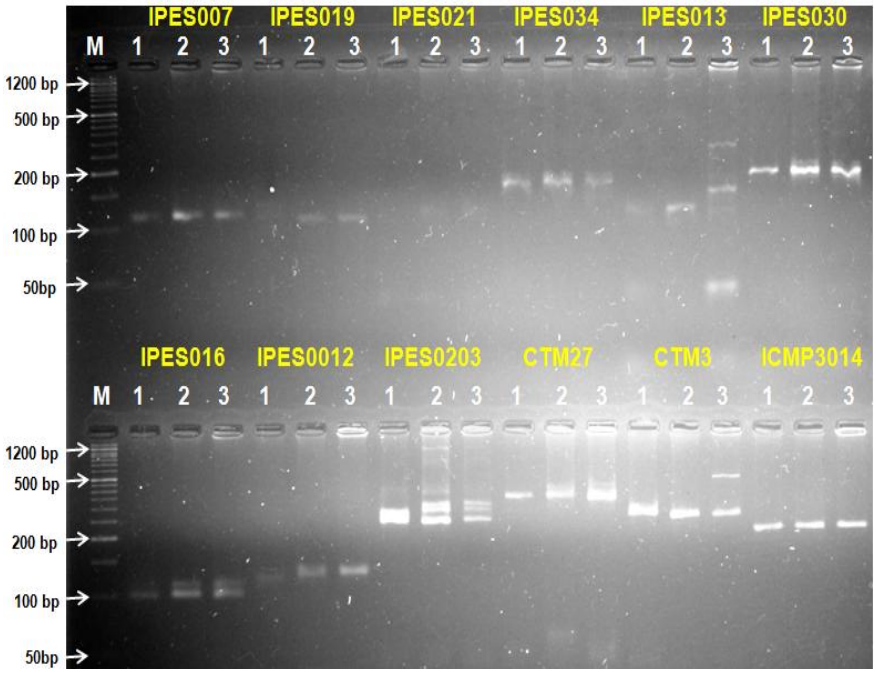

Fig 2.5 Gel photograph showing SSR allelic profile of MH2709 with 36 SSRs. M-50 bp DNA ladder; Lane 1- KBH108 (Check), 2- 86M86 (Check), 3- MH2709

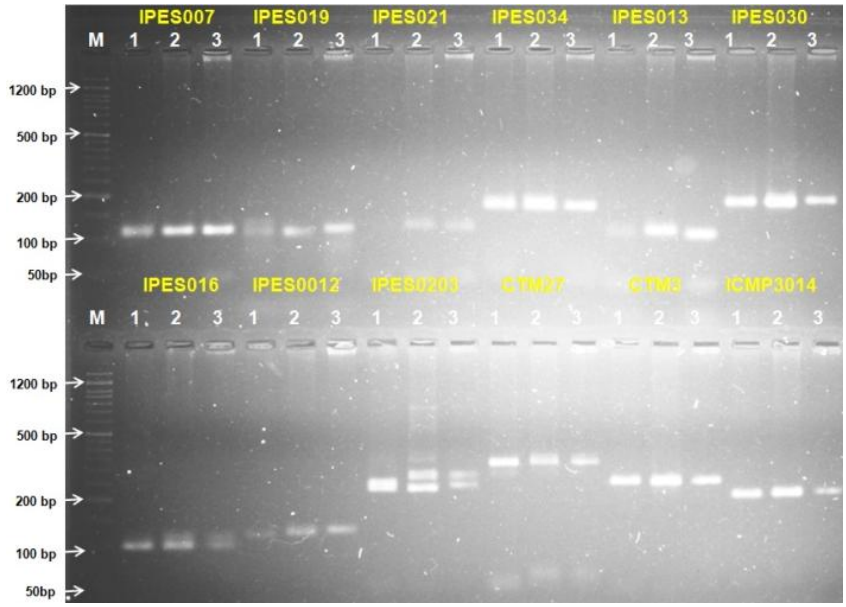

Fig 2.6 Gel photograph showing SSR allelic profile of MH2712 with 36 SSRs. M-50 bp DNA ladder; Lane 1- KBH108 (Check), 2- 86M86 (Check), 3- MH2712

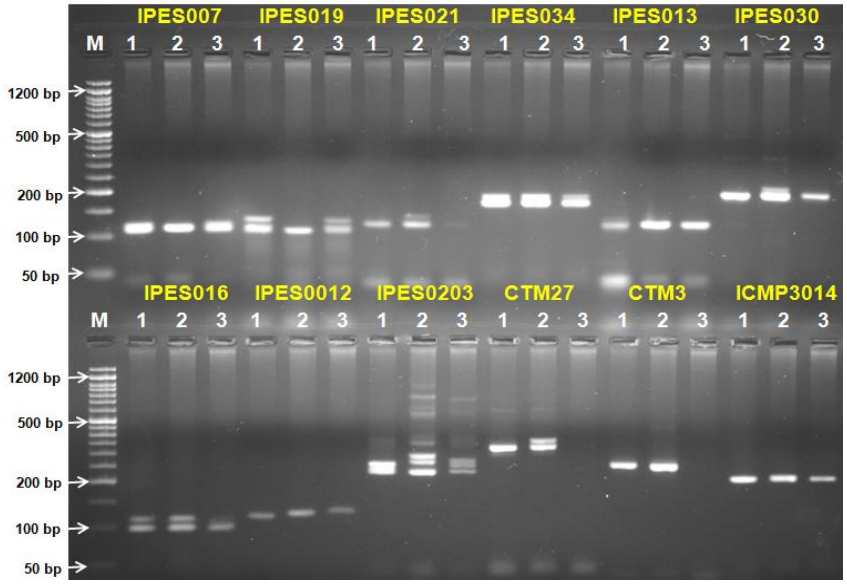

Fig 2.7 Gel photograph showing SSR allelic profile of MH2717 with 36 SSRs. M-50 bp DNA ladder; Lane 1- KBH108 (Check), 2- 86M86 (Check), 3- MH2717

Experiment 2: Molecular characterization of landraces in pearl millet

Plant material:

Young leaf samples of 2-3 leaf stage of high/low rancidity genotypes. A total of 24 pearl millet genotypes collected from NBPGR, Regional Station, Jodhpur were used for molecular characterization under this experiment (Table 2.1). Pearl millet has poor shelf life and rancidity is a major issue. To enhance its keeping quality there is a high need to address the issue of rancidity. Hence, screening of pearl millet landraces having low and high rancidity will be useful for developing superior genotypes.

Genomic DNA isolation and quantification

Good quality genomic DNA having sharp band was successfully isolated from fresh and young leaves of 24 genotypes of pearl millet as described in PMMB1 and quantified on agarose gel.

Table 2.1 List of genotypes used for molecular characterization

S. No.	Name of Genotype	Source
1	IC420347	NBPGR, Regional Station, Jodhpur
2	IC333179	NBPGR, Regional Station, Jodhpur
3	IC420368	NBPGR, Regional Station, Jodhpur
4	IC420371	NBPGR, Regional Station, Jodhpur
5	IC329045	NBPGR, Regional Station, Jodhpur
6	IC329047	NBPGR, Regional Station, Jodhpur
7	IC329055	NBPGR, Regional Station, Jodhpur
8	IC329056	NBPGR, Regional Station, Jodhpur
9	IC537960	NBPGR, Regional Station, Jodhpur
10	IC420312	NBPGR, Regional Station, Jodhpur
11	IC449438	NBPGR, Regional Station, Jodhpur
12	IC449444	NBPGR, Regional Station, Jodhpur
13	IC449463	NBPGR, Regional Station, Jodhpur
14	IC537954	NBPGR, Regional Station, Jodhpur
15	IC449472	NBPGR, Regional Station, Jodhpur
16	IC537955	NBPGR, Regional Station, Jodhpur
17	IC541016	NBPGR, Regional Station, Jodhpur
18	IC537984	NBPGR, Regional Station, Jodhpur
19	IC285152	NBPGR, Regional Station, Jodhpur
20	IC285200	NBPGR, Regional Station, Jodhpur
21	IC449435	NBPGR, Regional Station, Jodhpur
22	IC537970	NBPGR, Regional Station, Jodhpur
23	IC449446	NBPGR, Regional Station, Jodhpur
24	IC537958	NBPGR, Regional Station, Jodhpur

Table 2.2 Results of SSR primers used for PCR

Markers	No. of markers
Number of SSR markers used	118
Number of amplified markers	108
Number of non-amplified markers	10
Number of polymorphic markers	94
Number of monomorphic markers	14

Molecular characterization and SSR analysis

For PCR reaction, DNA was diluted accordingly to make available final concentration of 10 ng/µl and amplification reactions were carried out in a volume of 10 µl as described in PMMB1**.** A total of 118 SSR primers were used for PCR amplification and characterization among 24 pearl millet genotypes. Out of 118 SSRs, 108 primers amplified products of varying sizes ranging from 110 to 600 bp and 14 (11.9%) were monomorphic and 94 (79.7%) were

polymorphic (Table 2.2 and Fig. 2.8). A total of 238 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.53 alleles**.** Polymorphic Information Content (PIC) varied from 0.41 to 0.79 with an average of 0.58 PIC value.

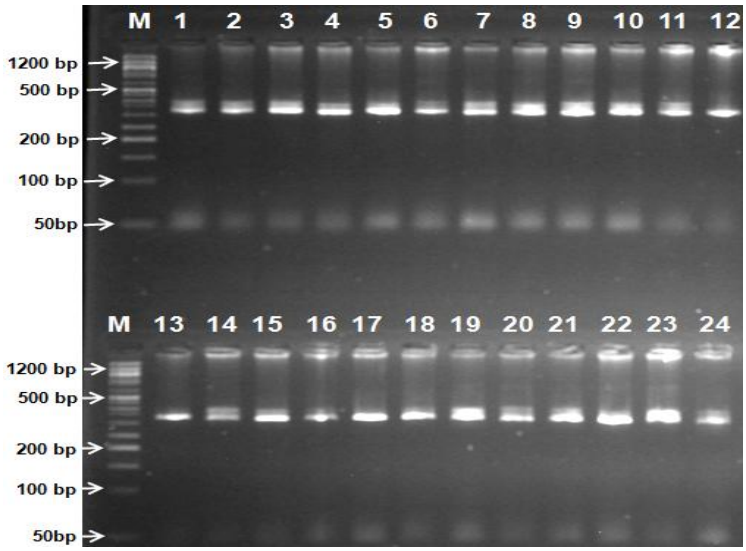

Fig. 2.8 Agarose gel showing amplification profiles of pearl millet genotypes using the primer CTM 12. Lane M-50 bp ladder, Lane 1-24 pearl millet lines

Diversity analysis and dendrogram construction

Differences in the DNA banding patterns were qualitatively scored from gel photographs for presence (1) and absence (0) of bands assuming that each band represents a unique genetic locus. Scoring was done for clear, unambiguous amplicons and their sizes were determined by comparing with 50 bp DNA ladder. Based on the presence or absence of amplicons, a binary 1-0 data matrix was created and used to calculate Jaccard's similarity coefficient (Jaccard, 1908). Cluster analysis was carried out among the genotypes based on Jaccard's similarity coefficients using UPGMA (Sneath and Sokal, 1973) and SAHN-clustering algorithm in NTSYS-pc, version 2.02e (Applied Biostatistics) software.

The genetic relationships among the genotypes were consistent and in complete agreement with the available data. The cluster analysis based on SSR markers discriminated well between the genotypes and gave 5 major clusters viz., I, II, III, IV and V with similarity coefficient ranging between 0.58 to 0.76 (Fig. 2.9). Cluster I contained eight genotypes and grouped together at similarity index of 0.64. In this cluster, pearl millet genotypes IC420347, IC420368, IC420312, IC537954, IC537984, IC449472, IC537955 and IC537970 are clustered together. Cluster II was obtained at a similarity index of 0.65 containing four genotypes namely IC537958, IC285152, IC285200 and IC420371. In this cluster, IC285200 and IC420371are grouped together at a genetic distance of 0.72.

In cluster III, five genotypes IC333179, IC449438, IC329056, IC329047 and IC329045 clustered together at a similarity index of 0.63. Here, IC333179 and IC449438 showed a more close similarity at a genetic distance of 0.73 while IC329047 and IC329045 exhibited similarity at a genetic distance of 0.68. Cluster IV included six genotypes viz. IC449435, IC329055, IC541016, IC449463, IC449446 and IC449444 and was obtained at a

similarity index of 0.62. IC537960 was entirely separated from all the genotypes and clustered in group V at a similarity index of 0.53. It has been proved that SSRs can be suitable and efficient tool for molecular characterization of many plant species including pearl millet. The pearl millet genotypes have been successfully characterized and the information revealed through this study is very useful and can be further used in breeding programmes.

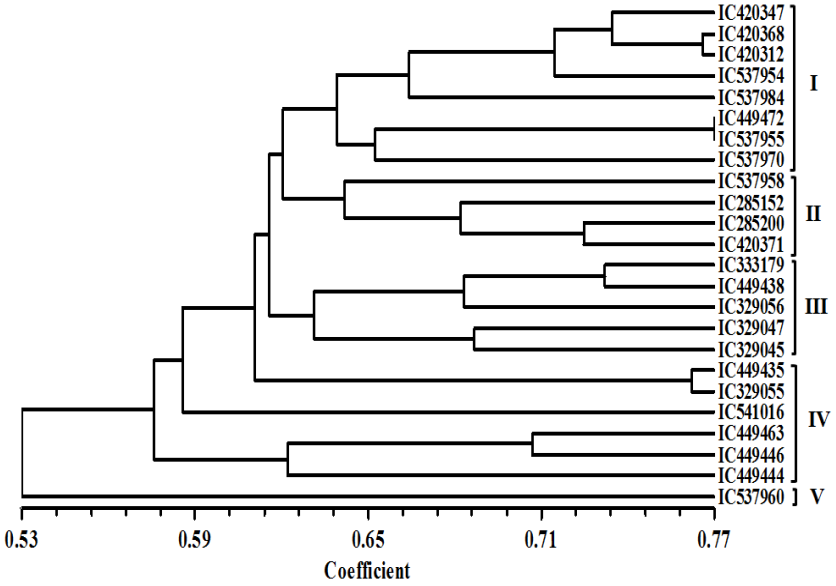

Fig. 2.9 UPGMA dendrogram showing genetic relationship among pearl millet genotypes based on Jaccard's similarity coefficients using SSR markers