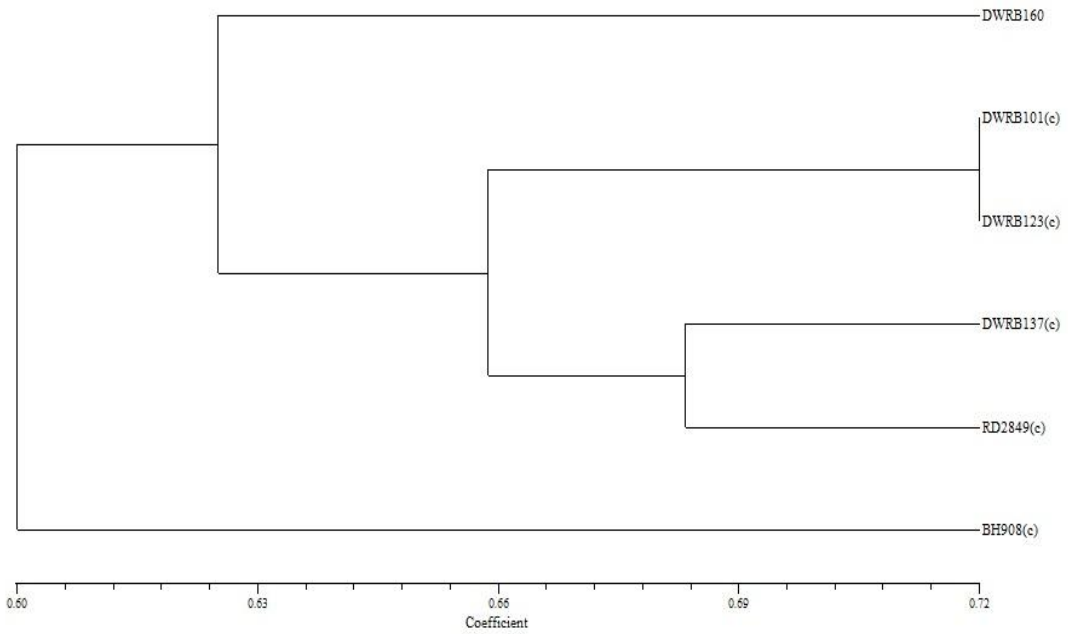
Molecular Report - AVT Final Year Trials (2018-19)

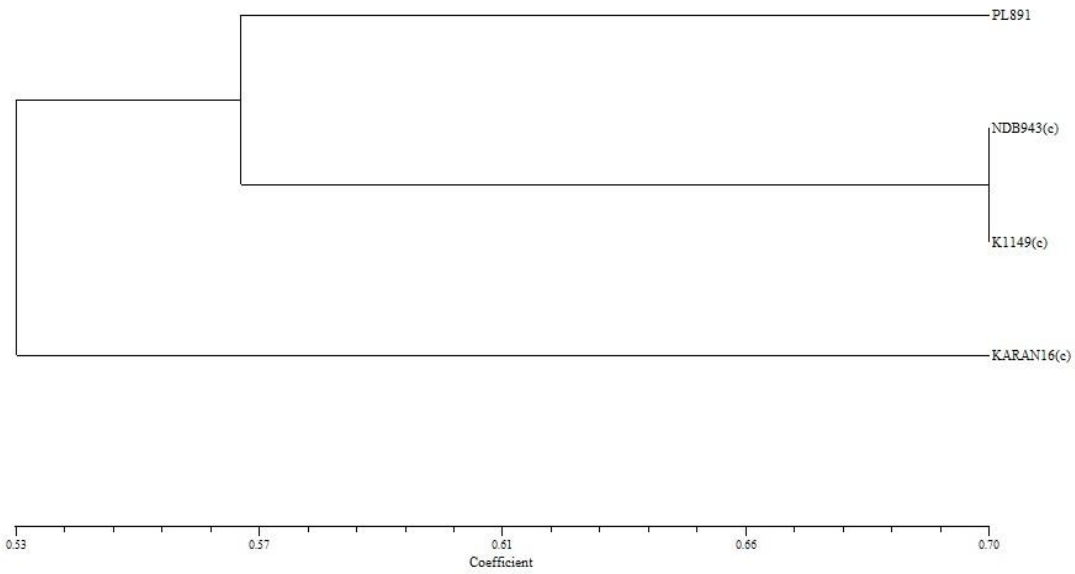
Test entries were characterized at molecular level to analyze genetic variability in final year advanced varietal trials 2018-19. A set of ten genotypes including two test entries (DWRB160 & PL891) and their respective checks (DWRB101, DWRB123, DWRB137, RD2849 & BH902 for DWRB160 and KARAN16, NDB943 & K1149 for PL891) were screened using barley specific molecular markers. Total 46 SSR/STS markers covering seven chromosomes of barley were screened to develop molecular profiles. Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus and polymorphism information content (PIC) were determined. Total 80 alleles were scored on PCR based amplification profiles for ten genotypes. The number of alleles ranged from 1 to 3 with an average of 1.76 alleles per locus. The band fragment size varied from 90 bp to 1500 bp with PIC values ranging from 0.0 to 0.75.

Allele molecular weight data of amplified profiles were converted to develop binary format (allele presence = “1” and allele absence = “0”) for genetic diversity analysis with NTSYS-PC version 2.1. The similarity matrix developed was used to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method of Arithmetic Means (UPGMA) to infer genetic relationships. For estimating the similarity matrix, null alleles were treated as missing data to reduce the biased genetic or similarity measures. The dendrogram were developed for each of two test entry and their respective check lines. These genotypes grouped within similarity coefficient (GS) value around 0.60 to 0.70 and showed sufficient genetic variability at molecular level. In both dendrogram, final year entries are placed at separate node thus distinguishing from their check lines, respectively.

The eventual intend of this effort is to develop molecular markers based amplification profiles for varietal characterization and to assess the level of genetic diversity in Indian barley.



UPGMA based clustering of NWPZ malt barley AVT final year trial (2018-19) entry and its respective checks for SSR/STS markers based amplification profiles



UPGMA based clustering of hulless barley AVT final year trial (2018-19) entry and its respective checks for SSR/STS markers based amplification profiles

Molecular Profiles of Barley AVT Final Year Trials (2018-19)

SN	Marker	Chr	DWRB160	DWRB101 (c)	DWRB123 (c)	DWRB137 (c)	RD2849 (c)	BH902 (c)	PL891	KARAN16 (c)	NDB943 (c)	K1149 (c)
1.	**Bmac154**	1H	130	130	130	130	130	130	130	130	130	130
2.	**Bmac213**	1H	180	180	168	168	180	168	180	180	168	168
3.	**Bmag382**	1H	109	109	109	109	109	109	109	109	109	109
4.	**Bmag579**	1H	126	126	126	126	126	126	126	126	126	110
5.	**MGB402**	1H	260	260	260	260	260	260	260,240	260	260,240	260
6.	**ScSSR10477**	1H	140	150	200	140	150	140	200	150	150	150
7.	**HvHVA1**	1H	136	136	136	136	136	136	136	136	136	136
8.	**Bmac175**	2H	180	180	155	180	180	155	180	155	155	180
9.	**EBmac640**	2H	190	176	190	176	190	190	190	176	176	190
10.	**Bmag15**	2H	181	181	181	181	181	181	181	181	181	181
11.	**EBmac525**	2H	125	149	149	149	149	149	149	149	125	125
12.	**EBmac623**	2H	168	168	154	154	154	168	168	154	154	154
13.	**cMWG658**	2H	580	580	580	600	580	600	600	600	600	600
14.	**Ebmatc39**	2H	170	170	170	150	150	150	150	150	150	150
15.	**Bmag006**	3H	274	274	274	274	274	274	274	274	274	274
16.	**Bmag603**	3H	122	122	122	122	122	140	122	140	122	122
17.	**Bmag877**	3H	165	165	153	153	165	165	165	165	165	165
18.	**Ebmac541**	3H	106	106	106	106	106	106	140	140	106	120
19.	**MWG 847**	3H	345	345	345	345	345	345	345	345	345	345
20.	**Bmag225**	3H	185	185	185	185	185	165	185	185	185	185
21.	**HvLTPPB**	3H	200	216	200	216	216	200	216	200	216	200
22.	**Bmag841**	3H	125	125	125	125	115	125	115	125	125	115
23.	**ABG500**	4H	189	189	189	189	189	189	189	189	189	189
24.	**HVM40**	4H	150	150	160	160	160	150	160	160	150	150
25.	**HVM67**	4H	136	136	136	136	136	126	136	136	126	126
26.	**HvMLOH1A**	4H	185	185	175	185	185	175	175	185	175	175
27.	**Ksug10**	4H	1500	1500	1500	1500	1300	1300	1500	1500	1500	1500
28.	**MWG634**	4H	800	800	800	800	800	800	800	800	800	800
29.	**WG622**	4H	161	161	161	161	161	161	161	161	161	161
30.	**Bmag353**	4H	119	90	90	119,90	90	90	90	119	119	119
31.	**Bmag337**	5H	145	165	145	165	145	145	145	145	165	165
32.	**Bmag751**	5H	189	189	189	189	189	189	189	189	189	189
33.	**Bmag812**	5H	167	157	157	167	167	167	167	157	167	147
34.	**GMS61**	5H	145	145	135	145	145	145	135	145	145	135
35.	**Bmac303**	5H	138	119	119	119	138	138	119	138	119	119
36.	**ABG458**	6H	248	248	248	248	248	248	248	248	248	248
37.	**Bmac40**	6H	236	210	210	236	236	210	210	236	210	210

38.	**Bmac500**	6H	110	110	110	190	190	110	110	190	110	110
39.	**GBM1215**	6H	240	200	200	240	200	240	240,200	240	240	240,200
40.	**HVM11**	6H	175	175	175	150	175	175	175	150	150	175
41.	**MWG2029**	6H	245	245	245	245	245	245	245	245	245	245
42.	**ABC15864**	7H	167	167	167	167	167	167	167	167	167	167
43.	**Bmac64**	7H	155	140	140	140	155	155	140	155	155	155
44.	**Bmac162**	7H	187	200	200	200	200	200	187	187	200	200
45.	**Bmac167**	7H	184	195	195	195	195	195	195	184	184	195
46.	**Bmag110**	7H	135	145	135	135	145	145	145	135	135	145

Molecular weight of amplified fragments measured in base pairs and calibrated with 100 & 500 bp Ladder

Molecular Markers Used for molecular characterization of AVT Final Year Entries and their respective checks (2018-19)

Sr No	Marker	Chr	Sequence of PCR Primer (5'-3')	Amplification Conditions
1.	Bmac154	1H	CTGGGTGATGAATAGAGTTTC TATTCTTCAAAAGATGTTCTGC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @72C
2.	Bmac213	1H	ATGGATGCAAGACCAAAC CTATGAGAGGTAGAGCAGCC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @72C
3.	Bmag382	1H	TGAAACCCATAGAGAGTGAGA TCAAAAGTTTCGTTCCAAATA	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @72C
4.	Bmag579	1H	CCTAGATAAGGAACATAGCCA CAAAGACCCTAACTCATGTTC	1 cycle of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 1 cycle of 5 mins @ 72C
5.	MGB402	1H	CAAGCAAGCAAGCAGAGAGA AACTTGTGGCTCTGCGACTC	1 cycle of 3 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
6.	ScSSR10477	1H	CATGGGAGGGGACAACAC CGACCAAACACGACTAAAGGA	1 cycle of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 1 cycle of 5 mins @ 72C
7.	HvHVA1	1H	CATGGGAGGGGACAACAC CGACCAAACACGACTAAAGGA	1 cycle of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 1 cycle of 5 mins @ 72C
8.	Bmac175	2H	CTACACCCCTACCATATAAACA CCTCCCCACATACCTTGT	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
9.	EBMAC640	2H	CTCAGTGCGTTACCAGTGC CCTGTCATGCATAACCTATGG	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
10.	Bmag15	2H	TTGAGGGCTGAACACTTCG GCCCACTGTCAAGGACAATT	Touchdown' PCR: 18 cycles of denaturing 1 min @94C and extension 1 min @72C, with annealing for 30s with temp decreased 1C every second cycel from 69C to 60C. Continue 20 cycles for 1 min @94C, 1 min @55C, 1 min @72C. End with 5 min @72C.
11.	EBMAC525	2H	TGACAGTGTCTCCAGTAATGA GTTTGTTCTTTTGATTTTGTTG	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
12.	EBmac623	2H	CGAACATTGTCGTGTTAGTAA CTGTCATGCATAACCTATGG'	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
13.	cMWG658	2H	CCAAGAAGGCGAAGAAGGTCC CTCACTGCCCAGAGAAACAGC	STS annealing temperature 62-65oC
14.	Ebmatc39	2H	TAGTCTCTTCATTTATACCATCACC CATGCTGATCCCCCTTCT	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
15.	Bmag6	3H	TTAAACCCCCCCCCTCTAG TGCAGTTACTATCGCTGATTTAGC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
16.	Bmag603	3H	ATACCATGATACATCACATCG GGGGGTATGTACGACTAACTA	1 cycle of 3 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 55C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
17.	Bmag877	3H	AAAGCTCATGGTAGATCAAGA TAGTTTTCCCAAAAGCTTCTA	1 cycle of 3 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 55C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C

18.	Ebmac541	3H	ACGGATCTACTTTAGCTAGCA AAACAACCCCACACAATC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
19.	MWG847	3H	GTCTTGGCCAGCTACTCCCG CGCACCTGCACCAGAGGTC	STS annealing temperature 65-67C
20.	Bmag225	3H	AACACACCAAAAATATTACATCA CGAGTAGTTCCCATGTGAC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
21.	HvLTPPB	3H	TGCTGAGACGCTGAGTACGTTG CAAACTCACGATTCCTCTCAAAG	35 cycles of 1 min at 94 deg C; 1 min at 50 deg C; 2 min at 72 deg C; and a final extension step of 5 min at 72 deg C
22.	Bmag841	3H	GGAAAGTACTTCAAACCTGAA CTTACAAGATGATGAGAACGA	3 min 94C, 45 cycles of 1 min @94C, 1 min @55C, 2 min @72C, final extension of 10 min @72C. 25 microlitre reactions contained 125 nM of each primer.
23.	ABG500	4H	ATTAATCCGACCGTCACTGC ACGAACTCCTCGCTGCC	STS annealing temperature 58-60C
24.	HVM40	4H	CGATTCCCCTTTTCCCAC ATTCTCCGCCGTCCACTC	Annealing (30 s) temperatures were progressively decreased by 1oC every second cycle from 64C to 55C
25.	HVM67	4H	GTCGGGCTCCATTGCTCT CCGGTACCCAGTGACGAC	'Touchdown' PCR of 48 cycles of 94C for 1 min denaturing and 72C for 1 min extension. Annealing (30 s) temperatures were progressively decreased by 1C every second cycle from 64C to 55C. Annealing conditions of 1 min at 55C were maintained during the final 30 cycles. The reaction ended with a 5-min extension at 72C
26.	HvMLOH1A	4H	CCTCCCCTCTGATATGATAA GTACAGACGGTTTAATTGTCC	1 cycle of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 1 cycle of 5 mins @ 72C
27.	Ksug10	4H	GTCCAGCTTCAGCGAGTAC GTGTTGATGTCCTTGAGGCC	STS annealing temperature 60C
28.	MWG634	4H	GTGCTGGGTGGATTAAAAAAGAGGG GAACTAAAGATAGGCGGGAGTACTG	STS annealing temperature 60C
29.	WG622	4H	CTGCCTGTTGATTTTCCATG TTCACCTTGCCATGACGA	STS annealing temperature 60C
30.	Bmag353	4H	ACTAGTACCCACTATGCACGA ACGTTCATTAAAATCACAACTG	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
31.	Bmag337	5H	ACAAAGAGGGAGTAGTACGC GACCCATGATATATGAAGATCA	1 cycle of 3 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 55C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
32.	Bmag751	5H	CACTGCAAATATTAAAATGGA GATCTACTGGTCCATAGTTGC	3 min 94C, 45 cycles of 1 min @94C, 1 min @55C, 2 min @72C, final extension of 10 min @72C.
33.	Bmag812	5H	ATAGTTCTTTCAGGACCAATG GTCATATGGATCTCCAAAGAG	3 min 94C, 45 cycles of 1 min @94C, 1 min @55C, 2 min @72C, final extension of 10 min @72C.
34.	GMS61	5H	CACCTGTTCCGTCCCGTC AACCTCTTTTTTATCCCTCGC	STS annealing temperature 60C
35.	Bmac303	5H	CCTCCAAGATTAGATCTCTCTC CCGTATATTTAAGAAATGGTGA	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C

36.	ABG458	6H	CCGGTCGGTGCAGAAGAG AAATGAAAGCTAAATGGGCGATAT	STS annealing temperature 55-58 C
37.	Bmac40	6H	AGCCCGATCAGATTTACG TTCTCCCTTTGGTCCTTG	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
38.	Bmag500	6H	GGGAACTTGCTAATGAAGAG AATGTAAGGGAGTGTCCATAG	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
39.	GBM1215	6H	ATGACCAGAAAACGCCTGTC GGATTCTGCACACACGAGAA	3 min at 94 deg C; 45 cycles with 30 sec at 94 deg C, 30 sec at 60 deg C (touchdown of 0.5 deg C / cycle for initial 10 cycles - final annealing of 55 deg C for remaining 35 cycles), 30 sec at 72 deg C; and a final extension step of 5 min at 72 deg C
40.	HVM11	6H	CCGGTCGGTGCAGAAGAG AAATGAAAGCTAAATGGGCGATAT	1 cycle of 3 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 55C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C
41.	MWG2029	6H	CCAGTTATCCGAATCCGGAA GTGGTCAGGTACATACGAAT	STS annealing temperature 60C
42.	ABC15864	7H	GCATAAACGGGTGTAAGAGC CATCCAGTTCAGAGGATAGAGC	STS annealing temperature 60C
43.	Bmac64	7H	CTGCAGGTTTCAGGAAGG AGATGCCCGCAAAGAGTT	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
44.	Bmac162		CATGTGTTGAAATCAGTTTTG CCCTCTCTCTCTCTCTCTCTC	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.
45.	Bmac167	7H	CATTTCCACTTCAAAATATCC CCAAAGTTTGAGTGCAGAC	1 cycle of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 30 cycles of 1 min @ 94C, 1 min @ 55C, 1 min @ 72C, 1 cycle of 5 mins @ 72C
46.	Bmag110	7H	ACGAGGAGGGACTAGTACAC CCAACTATATTAACAAGGCTCA	1 cycle of 3 min @ 94C, 1 min @ 58C, 1 min @ 72C, 30 cycles of 30 secs @ 94C, 30 secs @ 58C, 30 secs @ 72C, 1 cycle of 5 mins @ 72C.

Protocol for developing Molecular profiles of AVT Final Year Trials 2018-19

Development of Molecular profiles: An equal number of fresh, young leaves (ten days old) of five plants from each of AVT were bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai-Maroof *et al*, 1984). A set of 46 SSR/STS molecular markers covering whole genome of barley was used to develop amplification profiles of genotypes. PCR reaction was conducted in reaction volume of 10 ul containing 1X PCR buffer. 200 m M dNTPs, 0.25 uM of primer, 2Mm mgcl2, 1 unit Taq polymerase and 50 ng template DNA . PCR amplification was performed using BIORAD S 1000 thermocycler. PCR products were resolved by electrophoresis on 2 % agarose gels (HiMedia) at 4v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100 or 500 bpDNA ladder (Invitrogen, USA) depending on the amplified fragments size and corroborated with the reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by re-amplification using the same primer pair in the same conditions. Gels were stained with ethidium bromide (0.5ug/ml). DNA banding patterns were visualized with UV light and recorded by imagining system (Syngene Synoptics Ltd. USA).