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Diversity in Indian barley (*Hordeum vulgare*) cultivars and identification of genotype-specific fingerprints using microsatellite markers

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Introduction

Barley, *Hordeum vulgare* L., is one of the principal cereal crop of the world. There is a recognized need to reliably distinguish varieties of crop plants and establish their purity as a prerequisite for any breeding programme (Russel *et al*. 1997; Matus and Hayes 2002). Assessment of the extent of genetic variability within cultivated crop has important consequences in plant breeding and conservation of genetic resources (Petersen *et al*. 1994). The systematic evaluation of the molecular diversity encompassed in barley genetic resources is a prerequisite for its efficient exploitation in breeding as well as for development of the strategies for optimal conservation of genetic diversity (Malysheva-Otto *et al*. 2006). It is particularly useful in the characterization of individual accessions and cultivars, in detecting duplication of genetic material in germplasm collection and as a general guide for the choice of parents for breeding hybrids. In the context of plant improvement, this information provides basis for making decisions regarding selection of parental combinations that will maximize gain for selection and maintain genetic diversity (Matus and Hayes 2002).

Traditionally, morphological characters have been used to evaluate distinctness, uniformity and stability, and to establish the description of a genotype. This method is thought to be often influenced by environmental conditions (Russel *et al*. 1997) as well as being labour intensive. Rapid molecular tests, eliminating crop-stage dependency have been developed. The techniques of DNA fingerprinting have been established and various DNA profiling methods are currently available. Multilocus fingerprinting methods based on the polymerase chain reaction (PCR) have been extensively used to study the relationships among varieties and cultivars of many different plants. The most informative polymorphic marker system currently available is microsatellite or simple sequence repeats (SSRs) (Tautz and Renz 1984). Microsatellites are particularly attractive for distinguishing cultivars, since the level of variation detected at microsatellite loci is higher than that detected with most other molecular assays (Saghai-Maroof *et al*. 1994; Becker and Heun 1995; Bowers *et al*. 1996; Powell *et al*. 1996; Struss and Plieske 1998; Davila *et al*. 1999; Pillen *et al*. 2000; Ivandic *et al*. 2002).

Although there are many studies on variability and diversity in barley using molecular markers, there is limited evidence about diversity in Indian barley accessions. Studies combining the traditional approaches and molecular markers have not been extensively applied in crop improvement programmes. To make optimal use of Indian germplasm for effective breeding, evaluation of genetic diversity appears essential. In the current investigation, we compiled data and analysed the genetic diversity of 69 Indian barley lines by using a combination of traditional approaches of morpho–physiological traits as well as using a set of 16 SSR markers to generate DNA fingerprints. Our long term goal is to determine the utility of molecular marker diversity as a tool for gene discovery and biologically meaningful classification of germplasm.

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Materials and methods

Seed material

Seeds of 69 barley varieties (table 1) were procured from Banaras Hindu University, Varanasi and Directorate of Wheat Research (Indian Council of Agricultural Research), Karnal, India. Single plant selections of each genotype were multiplied and used.

Evaluation of morpho–physiological traits of 69 barley varieties

Sixty-nine barley cultivars were grown under field conditions in three replications and data for different morpho–physiological traits were recorded.

DNA isolation and SSR primers

Leaves of 69 selected Indian varieties were harvested at seedling stage from fields and DNA was extracted by CTAB method (Saghai-Maroof *et al*. 1984). Sixteen SSR markers of which 12 (HVM 5, BMS 64, HVWAXY, HVCMA, BLYRCAB, BMS 30, BMS 02, BMS 32, BMS 18, BMS 40, HVACLI and HVLEU) and 4 (HVM 4, HVM 9, HVM 3 and HVM 7) were used, derived from published research of Russel *et al*. (1997) and Saghai-Maroof *et al*. (1994), respectively, and were synthesized at Operon Technologies, California, USA.

PCR amplification and fragment analysis

DNA amplifications were carried out in 25 μL reaction mixtures, each containing a final concentration of 50 ng template DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 1 mM dNTP mix, 20 μM of each primer and 1 U taq polymerase (Banglore Genie, Bangalore, India) using the following PCR profile in a PTC-200, Thermal Cycler Techne, UK. Thermal cycling parameters consisted of initial denaturation at 95°C for 5 min, annealing at 55°C–60°C for 30 s, repeated cycles 40 followed by final extension at 72°C for 10 min. The amplified products were separated on 3.5% agarose gel electrophoresis (Senior *et al*. 1998).

Statistical analysis

Cluster analysis was done using UPGMA (unweighted pair-group method with arithmetic average) algorithm on NTSYS-PC, version 1.70 (Rohlf 1992). The polymorphic information content (PIC) of each microsatellite locus was evaluated through allelic frequency (Varshney *et al*. 2007).

$$\mathrm{PIC} = 1 - \sum_{i=1}^{k} pi^2$$

Where k is the total number of alleles detected for a microsatellite and pi the frequency of the ith allele in germplasm. Hierarchical clustering of genotypes was undertaken using SPSS version 6.0 based on morpho–physiological traits (SPSS, Chicago, USA).

Results

Data for 69 Indian barley varieties on morpho–physiological traits such as plant height, ear attitude, waxiness, purpose of cultivation, 1000 grain-weight and disease resistance to yellow rust, spot botch, and aphid were compiled, along with their region of cultivation (table 1). Hierarchical clustering of these genotypes showed the extent of variability. Ten clusters were obtained at an ecludian distance of 3 (data not shown). We further accessed the genetic variability by using microsatellite markers, and mapped the results from the analysis of morpho–physiological variability to the results obtained from microsatellite analysis.

In allele observation by 16 microsatellites, a total 52 alleles were detected. The number of alleles per locus ranged from 1 (marker HVACLI) to 8 (marker BMS 40) with an average of 3.25 alleles. The microsatellite primer pair BMS 40 showed the maximum polymorphism with PIC value of 0.947234, and was able to differentiate eight varieties. It also provides a unique marker for varieties K 24 and Clipper (bands 280 bp and 310 bp, respectively) which were discriminated from other 67 accessions (figure 1). The marker BMS 40 revealed null alleles (no amplified products) in three varieties: BH 393, DL 3 and Karan 92. The other highly polymorphic primers were BLYRCAB (allele 6) and HVM 3 (allele 6) with PIC value 0.898292.

The dendrogram prepared through cluster analysis (figure 2) suggested a high level of diversity among 69 varieties. The varieties could be grouped into four major groups, which were further characterized into 10 small clusters. We also obtained 10 clusters on the basis of their agronomic characteristics. Three clusters (I, VI and IX) comprised of only one variety i.e., BH 393, Jyoti and K 24 and had genetic similarity 0.44, 0.48 and 0.36, respectively. These three varieties are recommended for cultivation in eastern Gangetic plains of India but differ in resistance to spot blotch, yellow rust and aphid. BH 393 is resistant to spot blotch and aphid, Jyoti is highly susceptible to spot blotch and aphid but resistant to yellow rust, whereas K 24 is susceptible to all the three. Therefore, BH 393 can be considered as a better donor parent. Cluster II comprised of 17 varieties, of which, 14 were grown in the western Ganetic plains of India. Two varieties (Sonu and BHS 169) are from hilly zone (HZ) and one (Azad) is from eastern Gangetic plains. All the members of cluster II displayed the genetic similarity 0.46. In cluster III, five varieties were present with genetic similarity of 0.57. Highest genetic similarity was obtained in cluster IV (0.65). This cluster included NDB 1173 and Narendra 3 and C 84 and RDB 1 that could not be distinguished by microsatellite primer pairs used. The genetic similarity in these varieties can also be substantiated by their similar morphological characters. Two varieties C 84 and RDB 1 showed susceptibility to yellow rust and spot blotch, while NDB 1173 and Narendra 3 were resistant for both diseases. The NDB 1173 and Narendra 3 also showed similarity for other agronomical characters such as plant height, ear attitude,

Table 1. Morpho–physiological traits of 69 barley varieties.

No.	Genotype	Region and sowing condition	Pedigree	Major characteristics	Height (cm)	Ear attitude	Waxiness	Feed/malt purpose	1000 grain wt	Disease resistance		
										Yellow rust	Spot blotch	Aphid
1	BH 393	Haryana (IR)	California Mariout / RATNA	Hulled, 6 row	105–110	Semi erect	NW	FB	35–42	S	S	S
2	Amber	UP (RF)	K12 / C294	Hulled, 6 row	100–110	Non-droop	W	FB	45.7	S	S	MR
3	Narendra 4	UP (IR)	RD2035 / DL470	Hulled, 6 row	60–65	Semi erect	W	FB	49–54	MR	MR	S
4	Ratna	NWPZ (RF)	Selection from local material	Hulled, 6 row	100–105	Non-droop	NW	FB	48.7	S	S	MR
5	Kedar	NEPZ, (IR)	BG 1 × K 17	Hulled, 6 row	100–105	Semi erect	W	FB	43.2	MR	S	MS
6	NDB 1173	NEPZ, NWPZ (IR) Saline soils	BYT-LRA- 3 (1994-95) / NDB 217	Hulled, 6 row	65–75	Semi erect	NW	FB	44.25	MR	MR	MS
7	BHS 169	NHZ (RF)	Kailash × Briggs	Hulled, 6 row	92–100	Droop	W	FB	48.00	R	S	S
8	Sonu	HP (RF)	EB 233 × Giza 117	Hulled, 6 row	100–105	Non-droop	W	FB	48.7	R	S	S
9	VLB 1	UP (RF)	NP 109 × HBL 62	Hulled, 6 row	110–112	Non-droop	W	FB	49.7	T	MS	S
10	RD 57	Rajasthan (IR)	RS17 / PRIOR	Hulled, 6 row	90–95	Non-droop	W	FB	42.00	S	S	T
11	RD 103	Rajasthan (IR)	RDB1 / K 18	Hulled, 6 row	82–90	Non-droop	W	FB	35.0	S	S	S
12	HBL 113	NHZ (RF)	Selection from Zyphzee	Hulled, 6 row	85–95	Non-droop	W	FB	42	R	S	MS
13	BH 75	Haryana (IR)	RD 150 × AHOR 31-68	Hulled, 6 row	80–85	Non-droop	W	FB	40	MR	S	MS
14	Narendra 3	UP (IR)	K425 / Jyoti	Hulled, 6 row	65–75	Semi erect	NW	FB	40–45	MR	R	MS
15	Dolma	NHZ (RF)	Selection from USA 115	Hull less, 6 row	98–105	Non-droop	NW	FB	42.2	MR	S	S
16	C 164	Punjab / Haryana (IR)	C 155 / C 141	Hulled, 6 row	95–100	Non-droop	W	FB	50.9	S	MS	R
17	DWR28*	NWPZ (IR)	BCU 73 / PL 172	Hulled, 2 row	80–100	Droop	NW	MB	54–58	R	MR	MR

Table 1 (*contd.*)

No.	Genotype	Region and sowing condition	Pedigree	Major characteristics	Height (cm)	Ear attitude	Waxiness	Feed/malt purpose	1000 grain wt	Disease resistance		
										Yellow rust	Spot blotch	Aphid
18	DL472	Not released NWPZ (IR)	DL260 / DL20	Hulled, 6 row	88.2	Erect	W	FB	30.7	R	R	S
19	PL172	PUNJAB (IR)	RD 178 × DW 472	Hulled, 6 row	86–98	Non-droop	W	FB	44	R	MR	MR
20	BG25	Haryana (IR)	C 138 × CN170	Hulled, 6 row	94–96	Non-droop	NW	FB	48.6	R	S	MR
21	Ballia Barley	UP (RF)	Pure Strain local	Hulled, 6 row	96–105	Non-droop	W	FB	48.3	HS	S	S
22	K24	UP (RF)	CN 294 / K 12	Hulled, 6 row	100–110	Non-droop	W	FB	50.4	S	S	S
23	VLB56	Uttrakhand (R)	MOROCCO / VLB 122	Hulled, 6 row	60–64	Semi droop	NW	FB	35–42	T	-	-
24	C50	UP (RF)	Pure Strain local	Hulled, 6 row	100–105	Non-droop	W	FB	41.2	S	S	S
25	C84	UP (RF)	Pure Strain local	Hulled, 6 row	100–105	Non-droop	W	FB	41	S	S	MS
26	K12	UP (RF)	Pure Strain local	Hulled, 6 row	100–105	Non-droop	W	FB	46.7	S	S	MS
27	DL3	NWPZ (R) Not released	C 164 / C 50	Hulled, 6 row	70–85	Erect	W	FB	26.2	S	R	S
28	HBL276	NHZ (RF)	HBL 243 / HBL 238	Hull less, 6 row	110–115	Erect	W	FB	25–30	R	S	S
29	RD2052	Rajasthan (IR) CCN infested soil	{CAPI CM 67 × SO 727} × PL 101}	Hulled, 6 row	97–105	Non-droop	NW	FB	49.4	MR	R	MS
30	RD31	Rajasthan (IR)	R517 / PRIOR	Hulled, 6 row	85–90	Semi erect	W	FB	37–40	T	MS	MR
31	Vijaya	Western UP (IR)	K 12 / C 251	Hulled, 6 row	100–105	Non-droop	W	FB	42.9	S	S	MS
32	PL 426	Punjab (IR)	KARAN 92 / PL 101	Hulled, 6 row	95–102	Non-droop	NW	FB	42.6	S	S	S
33	K 409	UP (IR-LS)	Jyoti x DL 65	Hulled, 6 row	94–100	Non-droop	W	FB	44–48	S	S	S
34	Narendra 2	UP (IR)	DL470 / RD2035	Hulled, 6 row	75–93	Semi erect	W	FB	36–39	MR	MS	MR
35	RDB 1	Rajasthan (IR)	Gamma ray mutant of RS 17	Hulled, 6 row	90–95	Non-droop	W	FB	46.4	MS	S	MS

Table 1 (*contd.*)

No.	Genotype	Region and sowing condition	Pedigree	Major characteristics	Height (cm)	Ear attitude	Waxiness	Feed/malt purpose	1000 grain wt	Disease resistance		
										Yellow rust	Spot blotch	Aphid
36	BG 105	Haryana (IR)	C 14× MONTLOCI	Hulled, 6 row	90–98	Non-droop	W	FB	54.9	S	S	S
37	Ranjeet	Punjab (IR)	BG 1× MEX 5-13	Hulled, 6 row	95–105	Non-droop	W	FB	40.5	S	MS	MS
38	Bilara 2	Rajasthan (IR) saline soil	RS 17× C 251	Hulled, 6 row	100–105	Non-droop	NW	FB	36.5	HS	S	S
39	PL 419	Punjab (RF)	PL 101 / PH 182	Hulled, 6 row	9–95	Non-droop	NW	FB	43.6	S	S	S
40	BH 87	NWPZ (IR) Not released	CD 40 × CN 51 local control	Hulled, 6 row	90–95	Semi droop	NW	FB	48–52	MR	S	MS
41	K 14	UP (R)	Pure Strain local	Hulled, 6 row	96–105	Non-droop	W	FB	43.1	S	S	S
42	Himani	NHZ (RF)	Atlas 54 / BHS 15-80 / Kailash	Hulled, 6 row	65–72	Droop	W	FB	21	S	S	S
43	K 141	UP (RF)	K 18 x IB 254	Hulled, 6 row	105–110	Non-droop	W	FB	49.2	HS	HS	S
44	Narendra 1	UP (IR)	Karan15 / P408	Hulled, 6 row	84–102	Semi erect	W	FB	35–37	R	MR	MR
45	NDB 1180	Not released	BHS265 / Karan18	Hulled, 6 row	45–50	Semi droop	NW	FB	39-42	T	MR	S
46	Karan 92	Not released	Azam (dwarf) 1 / K 125 / DL 85	Hulled, 6 row	85–95	Erect	W	FB	30–40.5	S	T	T
47	Karan 280	Not released	Azam (dwarf) 1 / K 125 / DL 85	Hulled, 6 row	80–95	Erect	SW	FB	36.5	S	T	T
48	RS 6	Rajasthan (IR)	RS 17 / NP 21	Hulled, 6 row	65–72	Erect	W	FB	20.5	S	MR	S
49	BCU 73	NWPZ,NEPZ (IR)	WUM 143	Hulled, 2 row	95–98	Non-droop	W	MB	55	MS	MR	S
50	RD 2035	NWPZ (IR)	RD 137 / PL 101	Hulled, 6 row	95–100	Non-droop	NW	FB	44.1	S	S	S
51	Azad	UP (RF)	K 12 / K 19	Hulled, 6 row	110–115	Non-droop	W	FB	49	S	S	MR
52	K 603	NEPZ (RF)	K 257 / C 138	Hulled, 6 row	100–105	Non-droop	NW	FB	45.5	R	S	MR
53	Karan 16	NWPZ (IR)	Azam (dwarf)1 × EB 7576	Hull less, 6 row	75–80	Non-droop	W	FB	36.6	S	S	MR

Table 1 (*contd.*)

No.	Genotype	Region and sowing condition	Pedigree	Major characteristics	Height (cm)	Ear attitude	Waxiness	Feed/malt purpose	1000 grain wt	Disease resistance		
										Yellow rust	Spot blotch	Aphid
54	Clipper	NWPZ (IR)	Introduction from Australia	Hulled, 2 row	90–95	Non-droop	W	MB	47.8	R	MR	S
55	K 560	NEPZ (RF)	K 404 / DL 479	Hulled, 6 row	100–110	Non-droop	NW	FB	46.4	R	MR	MR
56	RD 2552	NWPZ, NEPZ (IR)	PD 2035 / DL 472	Hulled, 6 row	95–100	Non-droop	W	FB	45.3	R	T	MR
57	Karan 15	Not released (IR)	RDB 1 / EB 20	Hulled, 6 row	60–65	Droop	W	FB	26	S	T	T
58	Karan 4	Not released (IR)	RDB 1 / EB 20	Hull less, 6 row	70–80	Droop	SW	FB	27	S	T	T
59	DL 88	PZ (IR-TS), NWPZ (IR-LS)	BG 1 / Mex 5-13	Hulled, 6 row	97–100	Non-droop	NW	MB	48.6	MS	S	MS
60	Manjula	UP (IR-LS)	K 4128 / SOHAN	Hulled, 6 row	130–140	Non-droop	W	FB	51.8	S	S	MR
61	K 551	NWPZ,NEPZ (IR)	P 464 / JYOTI	Hulled, 6 row	102–105	Non-droop	NW	MB	52.6	R	MR	MR
62	Karan 741	Not released (RF)	Karan 163 / Karan 165	Hull less, 6 row	82–90	Droop	W	FB	25	S	S	S
63	Lakhan	UP (RF)	K 12 / IB 26	Hulled, 6 row	117–125	Non-droop	W	FB	48.9	MS	MR	MR
64	Karan 521	Not released (IR)	Karan 3 / Jyoti	Hull less, 6 row	74–85	Non-droop	W	FB	27	S	S	S
65	C138	Punjab / Haryana (IR)	C 251 / T 4	Hulled, 6 row	110–115	Erect	W	FB	40	S	MR	S
66	Karan 19	Not released (IR)	Azam (dwarf)13 / Puskin	Hulled, 6 row	81–87	Droop	SW	FB	25	S	S	S
67	RD 2503	NWPZ (IR)	RD 103 / BH 153 // RD 2046	Hulled, 6 row	100–105	Non-droop	W	FB	49.3	MS	MR	MR
68	Jyoti	NEPZ (IR)	K 12 / C251	Hulled, 6 row	125–130	Non-droop	W	FB	47	R	S	HS
69	RD 2624	NWPZ (RF)	BL 2 / RD 2508	Hulled, 6 row	65–92	Semi droop	SW	FB	42	R	S	T

FB, feed barley; MB, malt type barley; W, waxy; NW, non waxy; R, resistant; S, susceptible; MR, mild resistance; MS, mild susceptible; T, tolerant; RF, rainfed; IR, irrigated; LS, late sown; TS, timely sown.

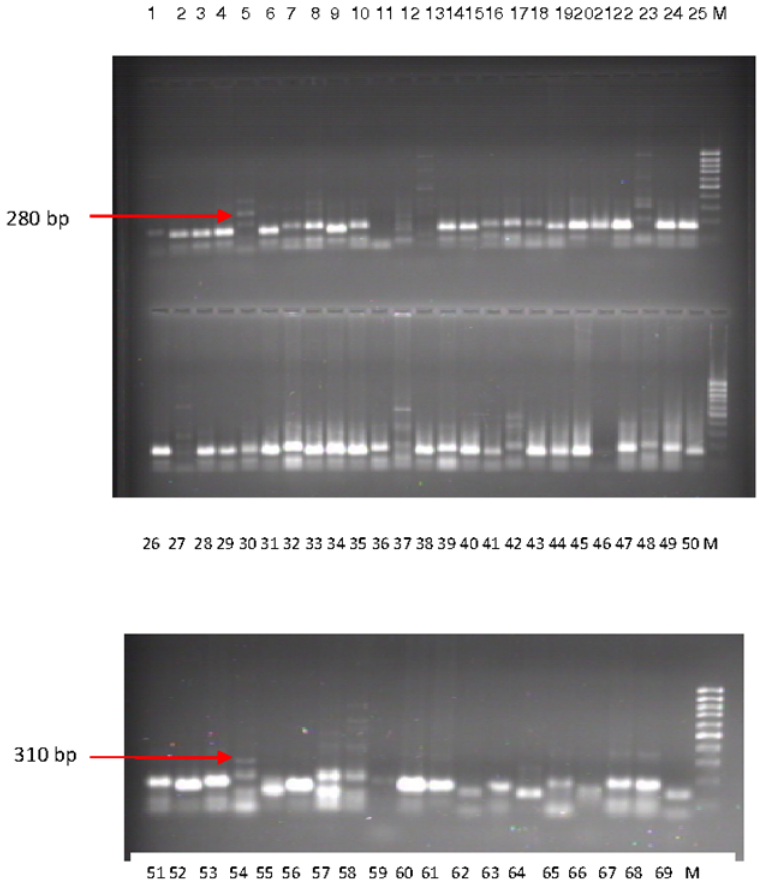

Figure 1. Microsatellite pattern differentiating among 69 varieties of barley as amplified with primer pair BMS 40; M is the 100-bp DNA marker (MBI Fermentas) 1, BH393; 2, Amber; 3, Narendra4; 4, Ratna; 5, K-24; 6, NDB1173; 7, BHS169; 8, Sonu; 9, VLB1; 10, RD57; 11, RD103; 12, HBL1; 13, BH 75; 14, Narendra 3; 15, Dolma; 16, C164; 17, DWR28; 18, DL472; 19, PL172; 20, BG25; 21, Ballia Barley; 22, Kedar; 23, VLB56; 24, C50; 25, C84; 26, K12; 27, DL3; 28, HBL276; 29, RD2052; 30, RD31; 31, Vijya; 32, PL426; 33, K409; 34, Narendra 2; 35, RDB1; 36, BG105; 37, Ranjeet; 38, Bilara 2; 39, PL419; 40, BH87; 41, K14; 42, Himani; 43, K 141; 44, Narendra1; 45, NDB1180; 46, Karan92; 47, Karan 280; 48, RS6; 49, BCU73; 50, RD2035; 51, Azad; 52, K603; 53, Karan16; 54, Clipper; 55, K560; 56, RD2552; 57, Karan15; 58, Karan4; 59, DL88; 60, Manjula; 61, K-551; 62, Karan 741; 63, Lakhan; 64, Karan 521; 65, C-138; 66, Karan19; 67, RD2503; 68, Jyoti; 69, RD2624.

leaf sheath, waxiness and 1000 grain weight. Cluster V, VII, VIII and X showed the genetic similarity values 0.53, 0.46, 0.44 and 0.34, respectively. The value of genetic similarity between varieties varied from 13% (BG 105 and BH 393) to 100% (Narendra 3 and NDB 1173, C 84 and RDB 1).

Discussion

The aim of the present investigation was to explore the genetic variability in Indian germplasm of cultivated barley using morpho–physiological traits and SSR markers. SSR markers are more specific because of the long primer sequences and high annealing temperature during PCR amplification. The characterization and assessment of genetic diversity among the barley genotypes would be important for designing breeding strategies for quantitative and qualitative traits. During the past five years an extensive amount of data have been produced concerning the evaluation of genetic diversity with SSR markers in different crops such as wheat sorghum, tomato, potato, rice, maize and barley. An overview of the reported results for barley (Maestri *et al*. 2002; Matus and Hayes 2002; Baek *et al*. 2003; Koebner *et al*. 2003; Malysheva-Otto *et al*. 2006) indicated that diversity parameters varied significantly among studies. We selected 69 cultivated barley (six rows / two rows) accessions from different regions of India, estimated the extent of variability in morpho–physiological traits important for adaptation, disease resistance, and yield, and surveyed microsatellite DNA polymorphisms at 16 loci to study the genetic divergence.

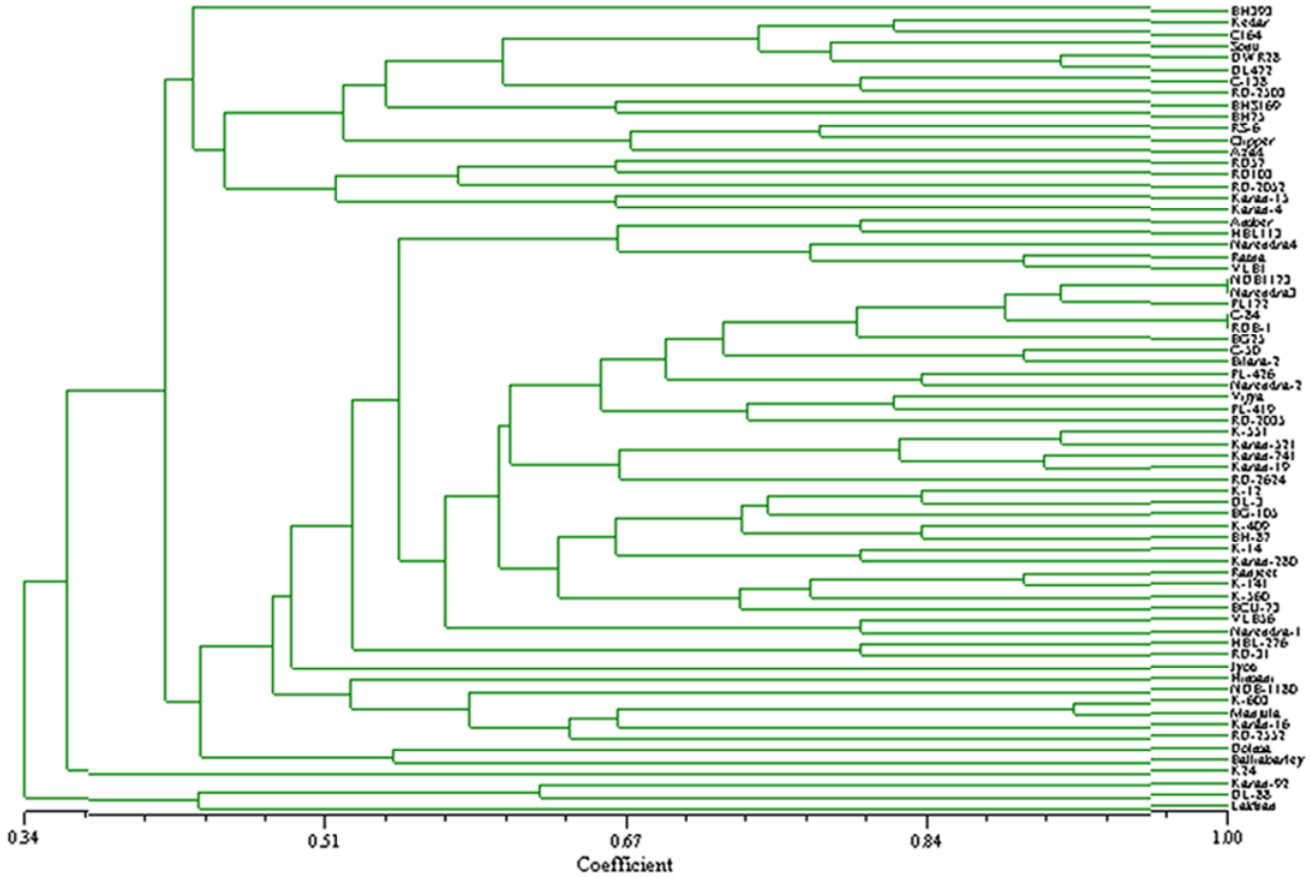

Figure 2. Dendrogram derived from banding pattern of microsatellite analysis of 69 Indian varieties of barley.

Genomic diversity was estimated according to allelic richness, i.e. number of detected alleles and occurrence of unique alleles.

A total of 52 alleles were observed from 16 SSR markers among all the selected 69 barley varieties used and the number of SSR alleles varied from one (HVACLI), to eight (BMS 40). The SSR marker (BMS 40) having di-nucleotide motif repeats $(CA)_{21}$, $(GA)_{21}$ amplified maximum number of alleles (eight) with highest PIC value of 0.947234 which proved that loci with larger number of tandem repeats are likely to have larger number of alleles (Struss and Plieske 1998).

Accessions K 24 and Clipper gave unique fragments of size 280 and 310 bp respectively at BMS 40 locus. Unique alleles are important because they may be diagnostic of a particular inbred line or for regions of the genome specific to a particular type of genotype (Senior *et al*. 1998). The occurrence of the unique allele is an indication of the diversity present in a germplasm and its potential as a reservoir of novel alleles for crop improvement (Matus and Hayes 2002). Similar to results reported by Matus and Hayes (2002) and Pillen *et al*. (2000), the highest PIC value (0.947234) was displayed by SSR marker (BMS 40) having highest number of alleles. However, same PIC value (0.947) was also reported by several workers for SSR markers varying in number of alleles (Saghai-Maroof *et al*. 1994; Struss and Plieske 1998; Matus and Hayes 2002). The UPGMA analysis for 16 SSR markers used in the study showed a high level of diversity among all the barley genotypes. All varieties represented mainly four major (I, II, III and IV) and 10 minor clusters. Similarly 69 varieties assembled in 10 groups after hierarchical clustering of their agro-morphological traits. This high level of genetic diversity among all barley genotypes may be due to their geographical pattern, agronomical characters, growth behaviour and different pedigree. This diversity leads to allelic richness that may represent various morphological traits in the population.

Assessing genetic diversity in 69 barley varieties of India using morpho–physiological traits as well as SSR markers gave overlapping results. It is concluded that the five primer pairs (BMS 40, BLYRCAB, BMS 30, HVCMA and HVM 3) could be used as a potent tool to discriminate Indian barley varieties investigated. Although further search can be made for even better SSR markers, the three or five primers obtained in this study provide an effective way to increase the efficiency of germplasm evaluation and to identify duplicate accessions in the barley germplasm collection.

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