Genetic Diversity Analysis Based on Random Amplified Polymorphic DNAs and Agro-morphological Traits in Barley

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The genetic diversity estimation of the released varieties and the improved germplasm is a prerequisite for their immediate exploitation in any crop improvement programme. Twenty two released varieties of barley in India and 14 exotic accessions including six cross derivatives of *H.vulgare* spp *spontaneum* and *H.vulgare* ssp *vulgare* were analyzed for genetic relatedness using RAPDs and the results obtained were compared with those obtained from the analysis of 17 agro- morphological traits. Four series (OPA, OPD, OPM, OPP) of random decamer primers were screened and 16 primers with polymorphic and reproducible banding pattern were selected. Four primers, namely, OPP15, OPP16, OPA7 and OPA8 together were able to identify most of the accessions. Relationships between the accessions were ascertained with the help of dendogram constructed by using UPGMA-clustering. RAPDs markers revealed high genetic diversity particularly among Indian released varieties. On the basis of RAPDs, the genotypes were clustered though loosely according to their place of breeding perhaps due to common ancestry. Morphologically, most of the Indian varieties were clustered into four groups. Clustering based on RAPDs and agro-morphological traits were incongruous. Genetic base of the Indian released varieties of barley is quite wide. The results provide valuable information on the suitability of RAPD for varietal fingerprinting of the released varieties and for the estimation of genetic diversity among accessions.

Key Words: Barley, Genetic Similarity, Genetic Diversity, *Hordeum vulgare* ssp *vulgare*, *H. vulgare ssp spontaneum*, RAPDs, Varietal Fingerprinting

Barley is one of the oldest food crops, ranking fourth in world acreage with an annual production of approximately 130 m tonnes (http://apps.fao.org/default.htm). A highly diverse gene pool is expected in barley as it is cultivated under diverse agroclimatic environment the world over. Information about the extent of genetic variation in the cultivated barley, *H. vulgare ssp vulgare*, land races of *H. vulgare* and its wild ancestors *H. vulgare* ssp *spontaneum* is essential for germplasm conservation and optimal designing of breeding programs. Assessment of genetic diversity in *Hordeum* has been carried out by a number of researchers using pedigree information (Martin *et al.*, 1991), morphological traits (Nevo *et al.*, 1979; Brown and Munday, 1982), biochemical markers (Doebley, 1989) and molecular markers (Tinker *et al.*, 1993, Melchinger *et al.*, 1994; Ordon *et al.*, 1997). The diversity analysis based on the morphological markers, pedigree record and biochemical traits have been questioned because of large unknown effects of environment (William et. al. 1997), unreliable pedigree records and unaccounted effects of selection, mutation and random genetic drift (Dweikat *et al.*, 1993) and also poor coverage of the genome, respectively. The random amplified polymorphic DNA (RAPD) has been proposed as a new source of genetic markers, which detects nucleotide sequence polymorphism by means of PCR using primer of arbitrary nucleotide sequence (William *et al.*, 1990; Welsh and McClelland 1990). It has several distinct advantages over RFLP like cost effectiveness, no use of radioactive element and requires no prior information for designing of the primers. It has proved valuable in the construction of the linkage maps, in the identification of the strains and varieties and in genomic diversity and phylogenic studies (Dawson *et al.*, 1993) in a wide array of taxa. In present study, exotic and indigenous accessions of *H.vulgare* ssp *vulgare* and the cross derivative of *H. spontaneum* and *H. vulgare* were subjected to RAPD analysis with the objectives of identification of cultivars/accessions and to estimate the extent of genetic diversity present and to group the accession on the basis of their similarity. The results were compared with those obtained on the basis of agromorphic traits.

Materials and Methods

The plant materials used in the study include accessions/lines/varieties of *H. vulgare* ssp *vulgare* and the cross derivatives of *H. spontaneum* and *H. vulgare* (Table 1). The *spontaneum* subspecies was represented by six

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Table 1. Name of the barley varieties/accessions along with their breeding center and special features

Sr. No.	Name of the varieties/ accessions	Pedigree and Origin	Key features
1.	BH 85	HAU, Hisar, India	Six rowed, released variety with medium height, medium maturity, medium dense spike with small and slender grain
2.	BCU 322	WI2291/H.spont.41-1, ICARDA, Syria	Ssp *spontaneum* type with erect seedling, medium height, medium flowering and two rowed medium dense spike
3.	BCU 344	H.spont.41-3/3/DEI RALLA06//BGS/RES1, ICARDA, Syria	Ssp *spontaneum* type with erect seedling, tall, medium flowering and six rowed
4.	BCU 383	H.spont.UNK-5, ICARDA, Syria	Ssp *spontaneum* type with spreading type of growth habit, two rowed and medium flowering.
5	BCU 768	CI 08887/CI 05761//Lignee 640, ICARDA, Syria	Exotic advance line, medium dwarf height, medium late heading, lax and six rowed.
6.	BCU2106	ATHS/LIGNEE 686, ICARDA, Syria	Exotic advance line with medium tall height , medium maturity and six rowed.
7.	Karan 741	AICBIP, Karnal, India	Released variety of medium height and medium maturity, six rowed and husk less
8	Ratna	Selection from local, IARI, New Delhi	Released six rowed non pigmented variety with tall plants and bold kernels
9	C-138	C251/T4, HAU, Hisar, India	Released six rowed non pigmented variety with lax, long ears and medium grains
10.	Manjula	K4126/Sohan, CSAUA&T, Kanpur, India	Released six rowed non pigmented variety, medium tall, early and hull less
11	Jyoti	K12/C251, CSAUA&T, Kanpur, India	Released six rowed non-pigmented variety, medium tall plant, and medium long and medium lax ears with golden grains.
12	K141	K18/IB 254, CSAUA&T, Kanpur, India	Released six rowed non pigmented variety with medium tall plants, medium lax ear and bold grains
13.	K 550	K1001/Sohan, CSAUA&T, Kanpur, India	Advance line non pigmented, medium early, medium tall and bold grains
14	BP1607	HAU, Hisar, India	Basal pigmented, spreading seedling, medium tall, medium maturity and lax spike
15.	Alfa 93	Aurora/Queen//Beka, DWR, Karnal, India	Released two rowed malt variety with basal pigmentation, medium height and late maturity
16.	Bilara-2	RS 17/C251, RAU, Durgapura, India	Released six rowed non pigmented variety, medium height, early maturity and bluish bold grains
17	DL-88	BG1/K572//K71/EB533, IARI, New Delhi, India	Released six rowed semi dwarf variety with early maturity and suitable for late sown condition.
18	RD2035	RD137/PL101, RAU, Durgapura, India	Released six rowed high yielding variety with medium height, medium maturity and medium grains
19.	RD 2052	AP1-CM-67/50-727//PL101, RAU, Durgapura, India	Released six rowed, high yielding non-pigmented variety, medium tall and yellow bold seed.
20	BCU 376	H.spont UNK-7, ICARDA, Syria	Exotic and H.spontaneum type with medium height, late flowering and two-row type, small and slender grain.
21.	RS17	Selection from local, RAU, Durgapura, India	Released six-rowed variety with tall plants, medium bold and yellow grains.
22.	BCU 1700	Shimla local, IARI, Shimla, India	Local Line, semi spreading seedling, medium tall, late in flowering, six rowed with lax spike
23.	BCU 1520	SLB 039-16, ICARDA, Syria	Improved germplasm, basal pigmentation, medium dwarf, late flowering, two rowed with lateral florets.
24.	Himani	Atlas 54/Kailash//BHS-15-88, IARI, Shimla, India	Released husk less pigmented six rowed variety with medium height and maturity with lax spike
25.	PL 56	Mutant of C164, PAU, Ludhiana, India	Released six-rowed variety with medium compact spike, medium bold grains and rough awn.
26.	BCU67	PITAYO/CAM/4/AVT/RM 150814//POR/DS/3/ CM 67/DUG2, ICARDA, Syria	Advance exotic line, spreading type seedling. Two rowed and lax spike
27	BCU 2940	Exotic introduction IARI, New Delhi, India	High lysine line, semi spreading, medium tall, medium grain with high protein content
28.	Victoria	Not known, Romania	Exotic introduction, with medium plant height and maturity and sterile tip on the spike and resistant to scald
29.	BCU369	H.spont.27-1//ER/APM, ICARDA, Syria	Interspecfic derivative, sub species spontaneum type, exotic, erect seedling, two rowed with small and slender grain.
30.	VLB-1	NP109/HBL 62, VPKAS, Almora, India	Released variety for hilly areas with dark green foliage, basal pigmentation, medium tall height and yellow grain.
31.	Rajkiran	RDB-1/Marocaine 079-CI 8334-Genesis// UUB72-282-2d-10d-7d, RAU, Durgapura, India	Released six rowed nematode resistant variety, medium early, medium height, medium dense spike and erect seedling.
32.	BHS 169	Kailash/Briggs, IARI, Shimla, India	Released six rowed variety for lower hills, non pigmented plant, medium dense spike, white awns and drooping ear heads
33.	Lakhan	K71/IB226, CSAUA&T, Kanpur, India	Released six rowed, non pigmented variety with medium tall plants, medium dense spike and bold grains
34.	Dolma	Selection from USA 115, Bhuntar, India	Released six rowed, non pigmented variety with lax spike, small, round husk less grains
35.	BCU 353	H.spont.41-3//WI2291/WI 2296, ICARDA, Syria	Exotic, interspecific derivative, non pigmented, two rowed, lax spike and slender grain
36.	BG105	C141/Montless, HAU, Hisar, India	Released six-rowed non-pigmented variety with medium height and early maturity and dense spike.

accessions which also included the cross derivatives. The 22 released indigenous varieties (both six and two row types) and 8 germplasm lines of exotic and indigenous origin maintained in Directorate of Wheat Research, Karnal's repository represented ssp *vulgare*. For each entry a random sample of 30 kernels was sown in pots under greenhouse. Leaf tissues from 18-day-old seedlings were collected and immediately processed for DNA extraction. DNA was isolated from 5-10 g of tissue by the modified CTAB procedure. The tissue was ground in liquid nitrogen and suspended in 20 ml of CTAB extraction buffer 2x (100 mM Tris, pH 8.0; 1.4 M NaCl, 20 mM EDTA, 2% 2 mercaptoethanol and 1.5% CTAB). The slurry was incubated for one hour at 60°C and extracted with an equal volume of chloroform/isoamyl alcohol (24:1). The aqueous phase was recovered after centrifuging at 17.000 rpm for 10 minutes. DNA was precipitated with an equal volume of isopropanol. The precipitated DNA was washed twice with 70% ethanol and dried under vacuum. The DNA pellets were then dissolved in the minimum of 10:1TE (10 mM Tris, 1 mM EDTA, pH 8.0). This was followed by the treatment with RNAase and extraction in phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1). DNA was reprecipitated by adding 1/10 volume of 3M sodium acetate and 2.5 volume of chilled ethanol and then washed in 70% ethanol. DNA was again vacuum dried and dissolved in 10:1 TE buffer. The quantification of DNA was done using flourimeter with calf thymus as the standard DNA and Hoechst 33258 as the fluorescent dye and also by running an aliquot in agarose gel along with the DNA of known concentration as standard.

RAPD analysis

Decamer oligonucleotides (Operon Technologies, USA) were used as primers for the present study. PCR was carried out in 25 μl reaction volume containing 3.5 mM of $MgCl_2$, 1 unit of Taq polymerase, 30 ng of genomic DNA and 0.5 μM of primer, 200 μmoles of each of dNTPs, 50 mM KCl and 10 mM Tris-Cl (pH8.3). The amplification were carried out in a Perkin Elmer thermal cycler programmed for initial denaturation at 92°C for 3 min., followed by 40 cycles of 1min for denaturation at 94°C, 1 min for primer annealing at 35°C and 1 min for amplification at 72°C. Amplified product were resolved on 1.4% agarose gel at constant voltage. After staining the gel with ethidium bromide, amplicons were visualized and photographed with the help of an ultraviolet transilluminator.

RAPD profiles were scored visually and the data were recorded as 1 and 0, with 1 depicting the presence of band and 0 indicating the absence. The fraction of band common between two genotypes was estimated using the formula of Nei and Li (1979). Only the clearly visible and reproducible bands were taken into consideration for the analysis. Cluster analysis, based on the unweighted pair group method with arithmetic mean (UPGMA) was carried out using NTSYS software programme to produce phenogram.

Agro-morphological data

Data were recorded in the field trial during the season 1995-96 for 17 characters viz., growth habit, basal pigmentation, auricle pigmentation, plant height, days to 75% flowering, lateral florets, spike density, row type, presence or absence of awn, awn roughness, tip sterility, waxiness of stem, seed shape, seed color, hulled/husk less, seed weight and seed protein content. The observation for each character was recorded as per the standard classification in barley used by NBPGR, New Delhi and these along with the center of origin were analyzed by using the NTSYS programme.

Results and Discussion

Thirty eight primers of the four series viz., OPA, OPD, OPM and OPP (Operon) were screened for amplification. Extensive polymorphisms (2-8 polymorphic bands) among the barley lines was detected with a number of primers. Of these, 16 primers giving good polymorphic and reproducible and discernible banding pattern were selected. Details of the primers used and the amplicons obtained are presented in Table 2. A total of 117 amplified products

Table 2. List of arbitrary primers and the extent of polymorphism in barley

Primer	Total number of bands	Polymophic bands	Per cent polymorphism
OPP3	5	2	40
OPP4	7	3	42.8
OPP6	5	2	40
OPP7	7	3	42.8
OPP8	7	4	57.1
OPP9	7	5	71.4
OPP11	5	2	40
OPP12	7	5	71.4
OPP13	9	5	55.5
OPP14	8	5	62.5
OPP15	10	8	80
OPP16	9	8	88.8
OPA4	9	6	66.6
OPA7	9	7	77.7
OPA8	6	4	66.6
OPA15	7	3	42.8

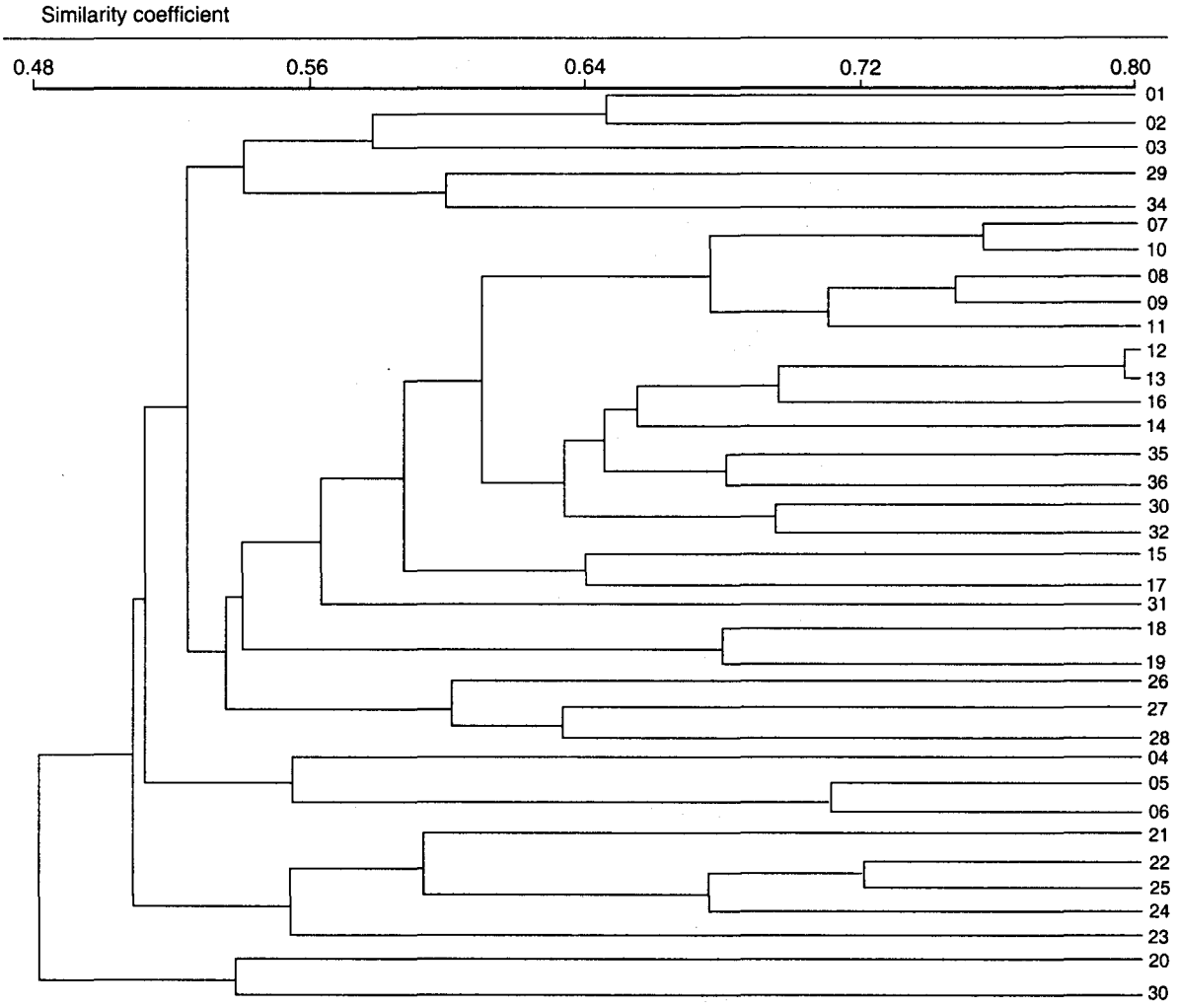

Fig. 1. Dendogram of 36 barley accessions based on RAPD markers

were obtained, of which 72 were polymorphic. Primers, OPP15, OPP16, OPA7 and OPA 8 together combination were able to identify all the varieties. Mean genetic similarity (MGS) of cultivars was estimated and presented in the Table 3. Genetic similarity estimates between the lines ranged from 0.392 to 0.796. Maximum genetic similarity was obtained between the lines K141 and K550 whereas the varieties BH85 and Lakhan showed the minimum similarity. Clustering of the lines based on UPMGA resulted in distinct groups (Fig. 1), which roughly corresponds to their center of breeding. Song and Robert (1995) also obtained broad correlation between the genetic diversity with geographic distribution. The indigenous varieties split into several groups and showed the maximum polymorphism. One group comprised Karan 741, Ratna, C-138, Manjula and Jyoti (nos 7, 8, 9, 10, 11) having the similarity value of 0.677 share more than 60% of the loci. The two varieties from Kanpur centre namely, K141 and K550 (nos. 12 and 13) though formed a separate subgroup with similarity coefficient of 0.80 but were closer in grouping with other released varieties from the Kanpur, center. The two varieties released from Durgapura center i.e. RD2035 and RD 2052 (Nos 18 and 19) are close to each other but appeared to be distinct from the rest of the released varieties of India. Maximum genetic distance was observed between an Indian released variety BH 85 and *H. spontaneum* accessions from ICARDA along with Indian released variety Lakhan and therefore, can be utilized for generating transgressive segregants. Lines like DL88, Himani, PL56 and Rajkiran were also distinct. Majority of the exotic material (nos. 2, 3, 4, 5, 6, 20, 23, 26, 27, 28, 29) did not form any separate cluster and were spread in different groups expectedly as most of the exotic lines selected for the present study were having diverse pedigree and were developed with different objectives.

Classification of the barley genotypes corresponding to row type was not revealed by the present study and it might be due to nonhomology of the primers included in the study to the site of row specific locus. The barley varieties developed and released in India have wide genetic base and it is still possible to morphologically differentiate them but the same may not be feasible in coming years with the release of more and more number of varieties and the convergence of phenotype towards a high yielding ideotype. It may, therefore, be necessary to employ newer molecular tools like STMS along with morphological traits for varietal authentication. The present study clearly indicates the value of RAPD markers in identification of the varieties. Four primers namely OPP15, OPP16, OPA7 and OPA8 could identify most of the accessions in the present study. Level of diversity revealed in the present study is quite large and was probably due to biased sampling as the varieties representing different breeding centers and accessions of species *H.vulgare* spp *spontaneum* were deliberately selected. Another interesting finding of the study is that the material developed at the Hisar Centre of India viz., nos 1, 9, 14, 36 was quite diverse whereas in the case of other breeding centres particularly Kanpur, the genetic base is quite narrow.

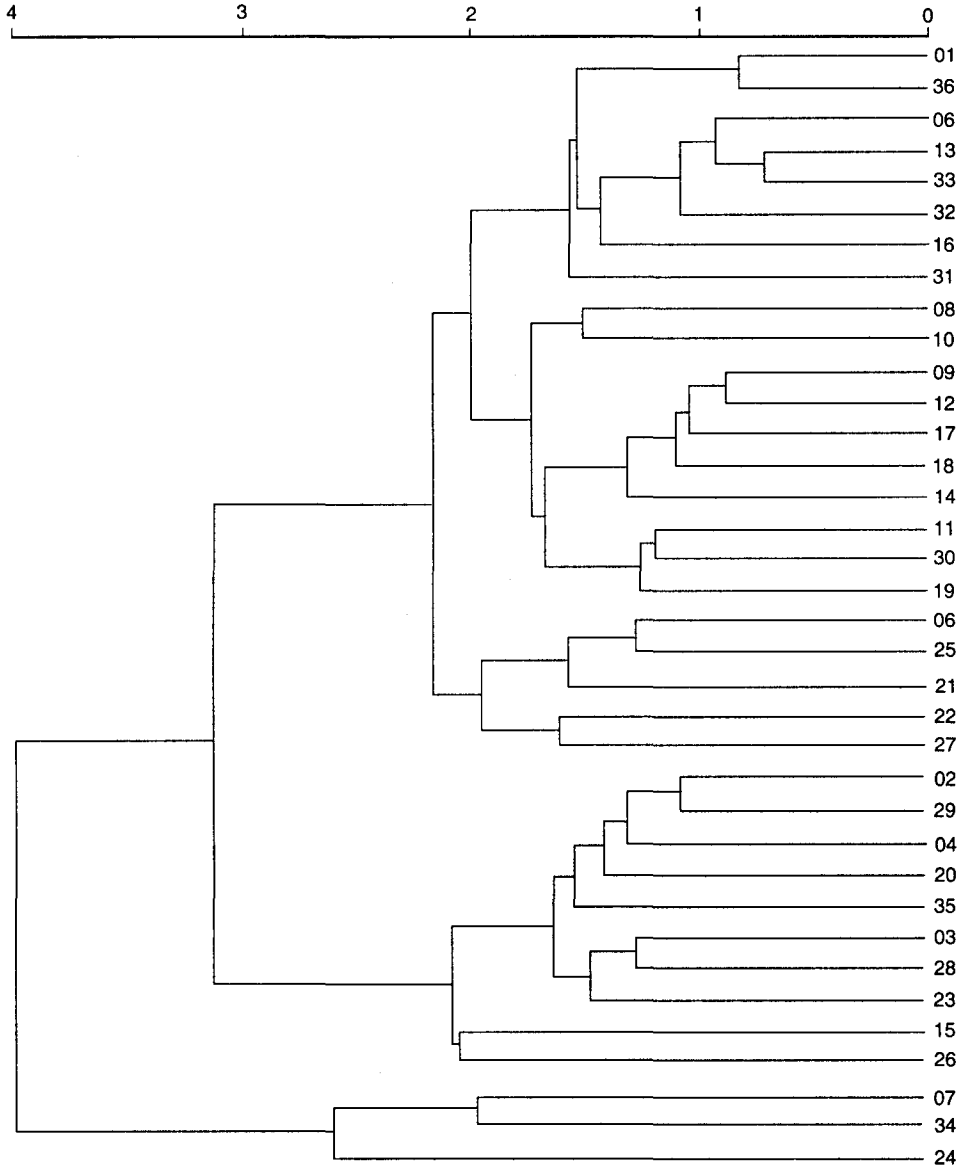

Fig. 2. Dendogram based on Euclidean distances of 36 barley accessions for agro-morphological traits

On the basis of the morphological descriptors, there were four distinct grouping (Fig. 2). The entries BH 85, BCU 2106, K550, Bilara 2, Rajkiran, BHS 169 and Lakhan (node nos., 1.6,13,16,31,32,33) were grouped under one large node. Another distinct group was of the entries Ratna, C138, Manjula, Jyoti, K141, BP1607, DL88, RD2035 and RD2052.This grouping was again more or less on the pattern of their center of development as it grouped three out of total six entries from Kanpur Centre. Third group was exclusively of the entries with exotic origin and includes mostly those developed at ICARDA. Morphologically most distinct line in the present study was BH 85 and Himani. There was not much congruence between the agromorphic data and RAPD markers but some broad link could be established between the two sets of data. BH 85 was both morphologically and on the basis of RAPDS markers the most distinct. The varieties from the Kanpur Centre form more or less a cohesive group under both sets of data. The loose parallelism between the two type of data might be due to the fact that genome fraction surveyed by the primers used in the study is random and may also involve the non coding regions of the genome and not specifically correspond to the functional part of the genomes only. Moreover, the numbers of morpho-physiological data collected in the present study were limited and not exhaustive. However, morphological and molecular survey of the released varieties in the present study indicates the genetic base of these varieties is not narrow and if used in the crossing programme along with the specific donors from the exotic sources like ICARDA in three way or double cross can still generate useful breeding material. The extent of genetic variation revealed in the indigenous material can be exploited for further improvement of barley first by selecting the parents on the basis of per se performance and then establishing their real divergent nature by DNA marker analysis. Further, this type of study will also clearly define the germplasm group and will help in assigning the line with unknown pedigree record to a particular group and thus should be of great help to the breeders in the selection of the parents. The divergent genotypes like BH 85, Lakhan, Himani DL88 and *H.spontaneum* accessions from ICARDA can be used in hybrid breeding programme too.

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