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Spatio-temporal genetic diversity in Indian barley (*Hordeum vulgare* L.) varieties based on SSR markers

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Barley is a popular cereal crop of nutritious and industrial importance and there is always a scope to improve a particular crop in view of its value and demand. Spatio-temporal studies discuss the diversity level among the existing varieties as well as the trend of genetic variation over a period of time and are useful in designing suitable strategies for genetic enhancement and improvement of a particular crop. Here, we used SSR markers to assess the level of spatio-temporal genetic diversity among fifty diverse released varieties of barley from India. Spatial diversity (0.233±0.16) was higher in varieties having exotic parentage as compared to the indigenous varieties (0.176±0.18) and significant diversity was also observed among the varieties released from different developing centers. There was a rising trend in % polymorphism and other diversity parameters among the barley varieties released before 1960s to 1970s, which may be attributed to the increasing usage of exotic germplasm during these decades. Increase in temporal diversity from 1970s to 1980s and to 1990s was not much as compared to earlier decades, which suggests that more diverse germplasm should be used to widen the genetic base of barley varieties released over the years. Further, structure and AMOVA results revealed strong differentiation among spatial groups, but not between temporal groups. The role of spatio-temporal genetic diversity studies and the importance of periodic monitoring of the varieties released for further crop improvement was emphasized.

Keywords: Nei's gene diversity, Polymorphism, Spatial diversity

Barley (*Hordeum vulgare* L.; Fam. Poaceae; self-pollinated, diploid, 2n=14) is popularly used as animal feed, food by poor people, and for malt production[1]. It is gluten free, palatable, easily digestible and nutritious (rich in vitamin B-complex and high-quality protein) food. Barley is classified as two-rowed and six-rowed barley[2]; hulled and hulless types. Two-rowed, hulled barley is preferred by malting industry (malt serve as raw material for malted food, beverages, etc. and its by-product is used as cattle feed), whereas hulless barley is used for food and making local beverages in tribal areas[3,4]. It is also consumed in the form of *sattu* and *lugri* (a fermented drink in the northern hills of India). It is primarily a crop of northern India with Rajasthan and Uttar Pradesh as the major barley producing states of India. Punjab, Haryana, Madhya Pradesh, Himachal Pradesh, Bihar, Uttarakhand, Jharkhand and Jammu & Kashmir are the other barley growing states[5].

In India, crop specific plant genetic resources available, and germplasm introduced through various exchange programs are used to produce improved crop varieties. In barley also, a number of introductions were made and varieties have been released[6] as direct introductions (Clipper from Australia in 1969; Alfa-93 from Argentina in 1995; BCU-73 from ICARDA in 1997, all three were two-rowed barley varieties with better malting quality, and Dolma, a two rowed hulless, was direct introduction as USA115 and released in 1974) or selection (HBL-113 as feed barley which is a selection from Zyphee from USA and released in 1995; Sonu is a selection from cross EB233/GIZA117) from exotic germplasm to improve the quality of barley varieties. A number of other barley varieties have been released[7] in India, in which exotic germplasm has been used as one of the parents viz. Ranjit, DL-88, BHS-169, Karan-16, BG-105, BH-75, RD-31, RD-57, Rajkiran, RD-2052, Geetanjali, etc. The present study included the varieties which have been released from 1945 to 1997 and may be categorized as varieties with indigenous origin and of exotic parentage; two-rowed and six-rowed; hulled and hulless, feed and malt type and the varieties having tolerance to salt and alkaline conditions (Azad, Bilara-2, DL-88) and also the first dwarf mutant barley variety RDB-1 released in India.

Genetic diversity studies are important in estimating the extent of genetic variation in the available varieties or germplasm. Genetic diversity allows populations to adapt to changing climate and its assessment help to tackle these changes by utilizing the available genetic resources[8-10]. Molecular markers like SSRs are the markers of choice for genetic studies due to their reproducible, multiallelic nature and high polymorphism rate[11,12]. SSR markers have been used[13-16] and are being used for varietal identification and genetic diversity[17-19]

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and genetic structure[20,21] studies in different crops. These markers have also been used in spatio-temporal kind of studies in barley and other crops[22-25].

In the present study too, we used SSR markers to assess the level of spatial and temporal genetic diversity among fifty diverse varieties of barley released from India.

Materials and Methods

DNA extraction and quantification

Fifty released varieties (Table 1) of barley were procured from [Directorate of Wheat Research (DWR), now known as ICAR-Indian Institute of Wheat and Barley Research, Karnal] for SSR

Table 1 — List of barley varieties, pedigree, development center and year of release used in spatio-temporal studies

Barley varieties	Pedigree	Developing center	Release year
RS-17	Selection from local barley	ARS (SKRAU), Durgapura	1960
RS-6	RS17/NP21	ARS (SKRAU), Durgapura	1970
RD-103	RDB1/K18	ARS (SKRAU), Durgapura	1977
RDB-1	Mutant of RS17	ARS (SKRAU), Durgapura	1971
BILARA-2	RS17/C251	ARS (SKRAU), Durgapura	1978
RD-2035	RD137/PL101	ARS (SKRAU), Durgapura	1994
RD-2503	RD103/BH153//RD2046	ARS (SKRAU), Durgapura	1997
RD-2508	RD 2035/P409	ARS (SKRAU), Durgapura	1997
RD-2052	Api-CM-67/SO-727// PL101	ARS (SKRAU), Durgapura	1987
RD-31	RS17/PRIOR	ARS (SKRAU), Durgapura	1977
RD-57	RS17/PRIOR	ARS (SKRAU), Durgapura	1977
RAJKIRAN (RD-387)	RDB1/ MORROCAINE	ARS (SKRAU), Durgapura	1979
C-84 (K-84)	Selection from local barley of Aligarh	CSAUA&T, Kanpur	1945
C-50 (K-50)	Selection from local barley of Sitapur (U.P.)	CSAUA&T, Kanpur	1950
BALLIA BARLEY	Selection from local barley	CSAUA&T, Kanpur	1956
K-12	Selection from local barley of Ballia	CSAUA&T, Kanpur	1956
K-14	Selection from local barley of Bahriach	CSAUA&T, Kanpur	1959
K-18	K12/K14	CSAUA&T, Kanpur	1963
K-19	K12/K14	CSAUA&T, Kanpur	1963
K-24	CN294/K12	CSAUA&T, Kanpur	1965
AMBER (K-71)	K12/CN294	CSAUA&T, Kanpur	1969
JYOTI (K 572/10)	K12/C251	CSAUA&T, Kanpur	1969
VIJAY (K-572/11)	K12/C251	CSAUA&T, Kanpur	1972
AZAD (K-125)	K12/K19	CSAUA&T, Kanpur	1975
K-141	K18/IB254	CSAUA&T, Kanpur	1982
JAGARATI (K-287)	C138/P103	CSAUA&T, Kanpur	1983
LAKHAN (K 226)	K71/IB226	CSAUA&T, Kanpur	1983
MANJULA (K-329)	K4126/SOHAN	CSAUA&T, Kanpur	1987
GEETANJALI (K-1149)$^{m/HLL}$	K12/K572/10//EB410	CSAUA&T, Kanpur	1991
KARAN-16^{HLL}	AZAD (DWARF) 1/EB7576	AICBIP, Karnal	1987
BCU-73 (Rekha)*/^{m}	WUM 143 (YAGAN)	**DWR, Karnal	1997
ALFA-93*/^{m}	AURORA/QUEEN/BEKA (Introd. from Argentina)	**DWR, Karnal	1995
C-164	C155/C141	Gurgaon (CCSHAU)	1962
C-138	C251/T4	Gurgaon (CCSHAU)	1956
BG-25	C138/CN170	CCSHAU, Hisar	1975
BG-105	C141 x Montlesso	CCSHAU, Hisar	1975
BH-75	RD150/AHOR 31-68	CCSHAU, Hisar	1983
HBL-316 (Gopi)	Mutant of HBL98	HPKV, Bajaura	1995
DOLMA^{HLL}	Selection from USA115	HPKV, Bajaura	1974
HBL-113*	Selection from Zyphzee	HPKV, Bajaura	1995
SONU (HBL-87)	Selection from EB233/GIZA117	HPKV, Bajaura	1980
KEDAR (DL-36)	BG1 x K-71	IARI, New Delhi	1979
RATNA (IB-226)	Selection from local material	IARI, New Delhi	1970
CLIPPER*/^{m}	Introduction from Australia	IARI, New Delhi	1969
RANJIT (DL-70)	BG1/Mex-5-13	IARI, New Delhi	1974
DL-88 (Malty)/^{m}	BG1/MEX5-13	IARI, New Delhi	1997
BHS-169	KAILASH/BRIGGS	IARI, Shimla	1987
PL-56	A mutant of C 164	PAU, Ludhiana	1975
PL-172	RD178 x DW 472	PAU, Ludhiana	1984
VLB-1	NP109/HBL62	VPKAS, Almora	1984

[*two-rowed barley; ^{m} malt barley; ^{HLL} hulless barley; ** now Indian Institute of Wheat and Barley Research]

genotyping, structure and spatio-temporal analysis. These varieties have been developed at various centers of All India Coordinated Wheat and Barley Improvement Project (AICW&BIP) viz., Himachal Pradesh Krishi Vishwavidyalaya HPKV, Bajaura (4); Chandra Shekhar Azad University of Agriculture and Technology (CSAUA&T), Kanpur (17); Directorate of Wheat Research (DWR), Karnal (3); Swami Keshwanand Agriculture Research Station, Rajasthan Agriculture University ARS (SKRAU), Durgapura (12); Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar (5); Indian Agriculture Research Institute (IARI), New Delhi (5); IARI, Shimla (1); Punjab Agriculture University (PAU), Ludhiana (2) and Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora (1). DNA was extracted from pooled leaf samples using CTAB method[26]. Purified DNA was quantified using DyNA Quant 200 fluorometer (Hoefer Instruments, USA) and 10 ng/µL working solution was prepared for polymerase chain reaction (PCR).

SSR genotyping

Fourteen SSR primer pairs were used to profile the selected fifty barley varieties. The PCR component concentrations were as follows: 25 ng of genomic DNA, 1.5 mM $MgCl_2$ (Applied Biosystems), 1U AmpliTaq Gold polymerase (Applied Biosystems), 1x PCR buffer without $MgCl_2$ (Applied Biosystems), 0.1 µM forward and reverse primers each and 0.2 mM of dNTP mix (Applied Biosystems). PCR reactions were carried out in a Perkin Elmer GeneAmp PCR system 9600 thermocycler. The 14 primer pairs used were from previous studies[27,28] and the PCR thermal cycle conditions followed were as per their protocol except that, the initial denaturation of 94°C for 10 min was applied as the enzyme used in our study was a hot-start AmpliTaq Gold polymerase. The amplified products were electrophoresed on 3% metaphor agarose gel and photographed using a Bio Imaging System (SynGene).

Data analyses

Alleles were scored for all the 14 SSR loci and PIC (Polymorphism Information Content) value was calculated[29] using the formula $1-\sum p_{ij}^2$, where p_{ij} is the frequency of j^{th} allele for i^{th} SSR locus. Since some of the loci consisted of more than two alleles per locus, so allelic data were converted to 0 and 1 depending upon the presence or absence of alleles and used to estimate Nei's gene diversity statistics using POPGENE version 1.32[30]. Jaccard's similarity coefficient was also calculated based on 0/1 matrix for UPGMA clustering using NTSYS-pc. ver. 2.1[31]. GenAlEx software[32] was used for Analysis of Molecular Variance (AMOVA). In addition, the software STRUCTURE[33] was used to investigate number of groups using a burn-in of 100,000 and a run length of 10,00,000 (admixture model). The number of sub-groups (K) was determined by running the program at different K values (1 to 10) with five independent runs for each K value. Peak value of delta K was calculated using Structure Harvester[34] to confer the number of distinct groups.

Results & Discussion

A total of 14 microsatellite loci were used to profile fifty barley varieties (Fig. 1) to assess their spatio-temporal genetic diversity. Fifty-five alleles were generated with number of alleles ranging from 2 to 7 with an average of 3.93 alleles per locus. PIC value ranged from 0.084 (HVBAKASI) to 0.740 (HVPRIB) with an average of 0.423 (Table 2). Nei's gene diversity ranged from 0.025 to 0.317 with an average of 0.21±0.17 and Shannon's information index varied from 0.062 to 0.480 with an average of 0.33±0.23.

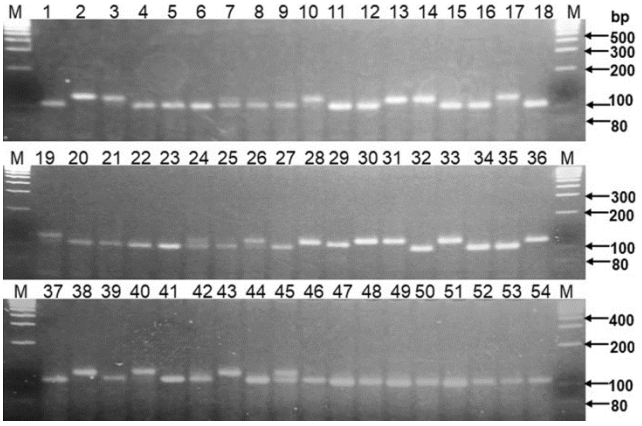

Fig. 1 — Gel picture of fifty Indian barley varieties with SSR primer HVM 60. [M is the 100 bp molecular weight marker and 1: Vijya, 2: BCU-73, 3: Dolma, 4: K-141, 5: Jyoti, 6: Ballia barley, 7: SONU, 8: Jagrati, 9: DL-88, 10: BH-75, 11: K-12, 12: K-18, 13: PL-56, 14: RD-31, 15: Manjula, 16: Lakhan, 17: Geetanjali, 18: K-24, 19: PL-172, 20: RDB-1, 21: RD-2503, 22: C-138, 23: K-19, 24: RD-57, 25: HBL-316, 26: Bilara-2, 27: VLB-1, 28: Rajkiran, 29: Karan-16, 30: Clipper, 31: Alfa-93, 32: C-50, 33: HBL-113, 34: Kedar, 35: Azad, 36: C-164, 37: BHS-169, 38: BG-105, 39: RS-6, 40: Ratna, 41: Amber, 42: K-14, 43: RS-17, 44: RD-2508, 45: Ranjit, 46: RD-2035, 47: C-84, 48: RD-103, 49: BG-25, 50: RD-2052 (Last four (51 to 54) varieties shown in the figure are not included in this study)]

Table 2 — Characteristics of SSR loci used for spatio-temporal studies in Indian barley varieties

Name of SSR locus	Putative function/map location	No. of alleles	PIC	h	I
HVM 3	Ribulose-1,5-bisphosphate carboxylase activase gene, Chr 4	4	0.578	0.25	0.40
HVM 40	Chr 4	3	0.568	0.29	0.46
HVM 62	Chr 3	5	0.623	0.20	0.32
HVBKASI	B-ketoacyl-acyl carrier protein synthase1 isoenzyme, Chr 2	4	0.084	0.02	0.06
HVCMA	α-amylase inhibitor, Chr 1	4	0.424	0.21	0.33
HVWAXYG	Starch synthase, Chr 1	5	0.633	0.24	0.37
HVCABG	Rubisco activase, Chr 4	6	0.550	0.19	0.30
HVELU	Thiol protease aleurin, Chr 7	2	0.320	0.29	0.46
HVPRIB	Pathogenesis related protein, Chr 1	7	0.740	0.23	0.36
HVM 68	Chr 4	5	0.346	0.17	0.27
HVM 60	Chr 3	3	0.474	0.28	0.41
HVM 27	Chr 3	3	0.183	0.17	0.30
HVSIPIA	SIP 1 gene	2	0.269	0.32	0.48
HVADHI	Alcohol dehydrogenase	2	0.136	0.23	0.37
Average		3.93	0.423± 0.20	0.209± 0.17	0.335± 0.23

[PIC: Polymorphism Information Content; h: Nei's gene diversity; I: Shannon's information index]

Table 3 — Spatio-temporal gene diversity parameters for Indian barley varieties based on SSR markers

Sample Type	% Pm	na	ne	h	I
Temporal gene diversity					
Varieties released before 1960s	29.09%	1.29±0.46	1.15±0.29	0.093±0.16	0.143±0.24
Varieties released in 1960s	50.91%	1.51±0.50	1.27±0.37	0.158±0.19	0.241±0.27
Varieties released in 1970s	78.18%	1.78±0.42	1.32±0.34	0.201±0.18	0.316±0.25
Varieties released in 1980s	70.91%	1.71± 0.46	1.34±0.37	0.204±0.19	0.316±0.26
Varieties released in 1990s	70.91%	1.71±0.46	1.33± 0.32	0.205±0.17	0.320±0.25
Spatial gene diversity					
Based on exotic pedigree or indigenous origin					
Varieties having indigenous origin	76.36 %	1.77±0.43	1.30±0.36	0.176± 0.18	0.280±0.25
Varieties having exotic germplasm as one of the parents/released as exotic introductions or selection of exotic germplasm	92.73 %	1.93±0.26	1.37±0.31	0.233±0.16	0.371±0.21
Based on developing centre					
ARS (SKRAU), Durgapur	67.27%	1.67±0.47	1.33± 0.36	0.198± 0.19	0.305±0.27
CSAUA &T, Kanpur	54.55%	1.54 ±0.50	1.20± 0.29	0.125± 0.16	0.201±0.24
*DWR, Karnal	50.91%	1.51±0.50	1.33±0.37	0.195±0.20	0.289±0.30
Gurgaon (CCSHAU) Centre	41.82%	1.42±0.50	1.30±0.35	0.173±0.21	0.253±0.30
CCSHAU, Hisar	47.27%	1.47±0.50	1.30±0.36	0.176±0.20	0.263±0.29
HPKV, Bajaura	58.18%	1.58 ±0.50	1.37±0.37	0.215±0.20	0.321±0.29
IARI, New Delhi	50.91%	1.51±0.50	1.31±0.38	0.178± 0.20	0.267±0.29
PAU, Ludhiana	30.91%	1.31±0.47	1.22±0.33	0.128± 0.19	0.187±0.28

[%Pm: % Polymorphism; na: observed number of alleles; ne: effective number of alleles; h: Nei's gene diversity; I: Shannon's information index; * now Indian Institute of Wheat and Barley Research**]**

Spatial diversity

The fifty barley varieties were divided into two groups, one containing varieties of indigenous origin and the other consisting of varieties which were released as exotic introductions or selections from exotic germplasm or having one of the parents of exotic origin. Observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I) and % polymorphism (1.93±0.26, 1.37±0.31, 0.23±0.16, 0.371±0.21, 92.73%; 0.177±0.43, 1.30±0.36, 0.176±0.18, 0.28±0.25, 76.36%) respectively, were higher in varieties with exotic parentage as compared to the ones having indigenous parents (Table 3). Barley varieties were also grouped into ten sets based on their development centers and Nei's genetic parameters were calculated (Table 3). Varieties from ARS (SKRAU), Durgapura showed highest % polymorphism (67.27%), followed by HPKV, Bajaura (58.18%) and CSAUA&T, Kanpur (54.55%) while the varieties from PAU, Ludhiana showed lowest %polymorphism of 30.91%. Varieties released from HPKV, Bajaura, followed by ARS (SKRAU), Durgapura and DWR, Karnal showed highest Nei's gene diversity and Shannon's information index (0.215±0.20, 0.321±0.29; 0.198±0.19, 0.305±0.27; 0.195±0.20, 0.289±0.30, respectively).

Temporal diversity

The temporal diversity was studied in the varieties released over a period of time based on decade groups [P1 (before 1960s), P2 (1960s), P3 (1970s), P4 (1980s) and P5 (1990s)] except P1, which consisted of varieties released during the 1950s and the only variety released in 1945. The following trend was observed in the % polymorphism, observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I) from P1 to P2 and to P3 [29.09%, 1.29±0.46, 1.15±0.29, 0.093±0.16 and 0.143±0.24 (P1); 50.91%, 1.51±0.50, 1.27±0.37, 0.158±0.19 and 0.241±0.27 (P2); 78.18%, 1.78±0.42, 1.32±0.31, 0.201±0.18, 0.316±0.25 (P3), Table 3]. And Nei's gene diversity (h), Shannon's information index (I) of P4 and P5 decades was 0.204±0.19, 0.316±0.26 and 0.205±0.17, 0.320±0.25 respectively.

AMOVA Analysis

Analysis of molecular variance (AMOVA) based on spatial and temporal distribution of varieties revealed more variation (95%) within decade groups compared to the variation among decade groups (5%). Similarly, 85% variation was contributed by varieties released from a particular center compared to 15% variation contribution due to varieties released among different developing centers. When the varieties were grouped based on their exotic and indigenous parentage, the variation was more within two groups (86%) than between group variation (14%) (Table 4).

UPGMA cluster analysis

UPGMA cluster analysis (Fig. 2) based on Jaccard's similarity coefficient, grouped the fifty barley varieties into two major clusters I and II. And

Table 4 — AMOVA analysis of Indian barley varieties based on SSR markers

Sample Type	Level of variation	df	SS	MS	Est. Var.	%
Temporal groups	Among groups	4	42.011	10.503	0.342	5%
	Within groups	45	323.369	7.186	7.186	95%
*Spatial groups (based on development centers)	Among groups	7	91.018	13.003	1.194	15%
	Within groups	40	263.649	6.591	6.591	85%
Spatial groups (based on indigenous and exotic origin)	Among groups	1	33.043	33.043	1.164	14%
	Within groups	48	332.337	6.924	6.924	86%

[*VPKAS, Almora (1variety) and IARI, Shimla (1 variety) not included in AMOVA analysis. df: degree of freedom; SS: sum of squares; MS: mean of squares]

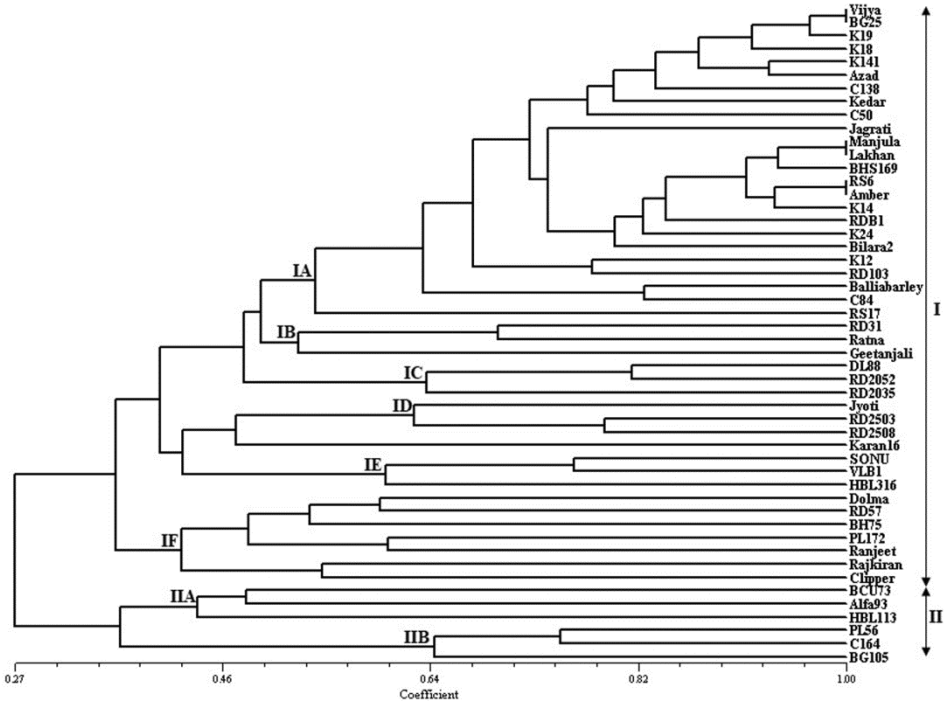

Fig. 2 — Cluster analysis of fifty Indian barley varieties based on SSR markers

cluster I was further divided into IA, IB, IC, ID, IE and IF and II in to IIA and IIB. IA consisted of 16 out of 17 varieties released from CSAUA&T, Kanpur. IB consisted of three varieties RD-31 (ARS (SKRAU), Durgapura), Ratna (IARI, New Delhi) and Geetanjali (CSAUA&T, Kanpur). IC and ID comprised of varieties released from ARS (SKRAU), Durgapura and DL-88, Jyoti and Karan-16. IE consisted of varieties released from the hill regions HPKV, Bajaura and Almora and two out of three are of exotic origin. Six out of seven varieties grouped in sub-cluster IF are exotic. Sub-cluster IIA comprised of two-rowed varieties having exotic origin. IIB consisted of PL-56 and C-164 and BG-105 and PL-56 (a mutant of C-164).

Structure analysis

Structure analysis revealed two groups GI and GII (Fig. 3). GI consisted of all the varieties which were released as direct introductions/selections from exotic germplasm or having one of the parents of exotic origin [Clipper, Alfa-93, HBL-113, BCU-73 (all two-rowed); Dolma, Karan-16, Geetanjali (all hulless), Ranjit, DL-88, BG-105, BH-75, RD-31, RD-57, Rajkiran, RD-2052] except BHS-169. All the malt barley varieties were also included in Group I. All the

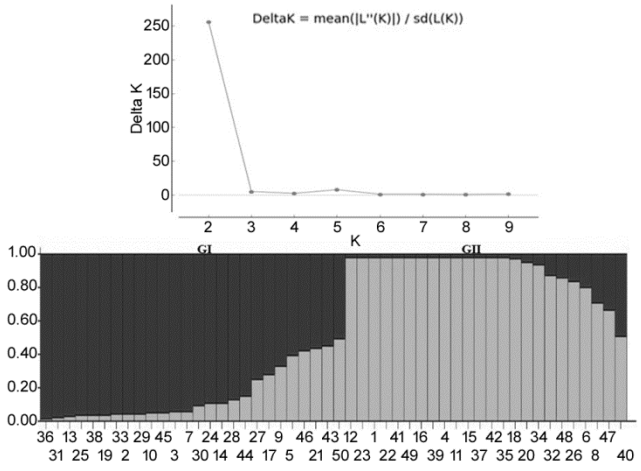

Fig. 3—Population structure in Indian barley varieties based on (A) Delta K value; and (B) GI and GII groups observed based on structure analysis. [1: Vijya; 2: BCU-73; 3: Dolma; 4: K-141; 5: Jyoti; 6: Ballia barley; 7: SONU; 8: Jagrati; 9: DL-88; 10: BH-75; 11: K-12; 12: K-18; 13: PL-56; 14: RD-31; 15: Manjula; 16: Lakhan; 17: Geetanjali; 18: K-24; 19: PL-172; 20: RDB-1; 21: RD-2503; 22: C-138; 23: K-19; 24: RD-57; 25: HBL-316; 26: Bilara-2; 27: VLB-1; 28: Rajkiran; 29: Karan-16; 30: Clipper; 31: Alfa-93; 32: C-50; 33: HBL-113; 34: Kedar; 35: Azad; 36: C-164; 37: BHS-169; 38: BG-105; 39: RS-6; 40: Ratna; 41: Amber; 42: K-14; 43: RS-17; 44: RD-2508; 45: Ranjit; 46: RD-2035; 47: C-84; 48: RD-103; 49: BG-25; 50: RD-2052]

varieties developed at Durgapura (except RS-6, RDB-1, RD-103 and Bilara-2); DWR, Karnal; PAU, Ludhiana; HPKV, Bajaura; CCSHAU, Hisar (except BG-25) and Almora were placed in Group I. Group II included all the varieties except two (Geetanjali and Jyoti) released from Kanpur; IARI, New Delhi and Shimla. All the varieties of Group II are of indigenous origin, six-rowed and hulled.

Spatio-temporal diversity studies of the existing varieties/germplasm based on molecular markers is encouraging as it reveals the increasing or decreasing trend of genetic variations over a particular period of time and their place of origin/development. The resulting information may help in making strategies for further exploration, collection, conservation and utilization of respective germplasm for crop improvement.

In this study, spatio-temporal diversity studies were carried out in fifty barley varieties based on SSR markers and fifty-five alleles were amplified with an average of 3.93 alleles per locus which is higher than the earlier reported[14] value of 2.4 alleles per locus in forty elite barley varieties from China, Canada, Australia, France, Germany, Japan, UK and USA, which probably may be due to the reason that the varieties and the SSR markers used in our study are genetically more diverse and polymorphic respectively. Our study revealed an average PIC of 0.423, which is less than but comparable to the PIC value of 0.58 from an earlier report[22] which included 504 European barley cultivars and showed the existence of a considerable amount of diversity in the Indian barley varieties included in this study.

Spatial diversity

Barley varieties with exotic parentage showed higher % polymorphism and Nei's genetic diversity parameters as compared to the ones having indigenous parents. Significant differences were also observed in % polymorphism and other diversity parameters among the varieties released from different centers. Further the varieties released from HPKV, Bajaura showed highest Nei's gene diversity and Shannon's information index, which may be due to the reason that that all the varieties except one have been released as direct introduction or selection from exotic germplasm. The more diverse nature of barley varieties with exotic parentage as compared to indigenous group is also evident from this study.

Temporal diversity

Genetic diversity was studied among different temporal groups. The increase in genetic differentiation parameters from P1 (before 1960s), to P2 (1960s) to P3 (1970s), may be due to the increasing trend of using exotic germplasm in barley breeding program. From P3 (1970s) to P4 (1980s) and to P5 (1990s)], there was a slight increase in Nei's gene diversity and Shannon's information index, but no significant loss of genetic diversity was observed in this set of barley varieties over a period of five decades. Previous study[22] in European barley cultivars also revealed no significant loss of diversity. So, there is a need to use more diverse germplasm in barley breeding programs to explore novel alleles and further enhance its genetic base as also reported by earlier genetic diversity study[16] on Brazilian barley.

UPGMA cluster analysis

UPGMA cluster analysis revealed clear separation of most of the varieties with exotic parentage; varieties released from CSAUA&T, Kanpur; ARS (SKRAU), Durgapura; DWR, Karnal; hill regions and two-rowed types with some exceptions. Close groupings of these varieties in different sub-clusters showed their relatedness.

AMOVA analysis and structure analysis

Any significant population structure attributed to temporal groups was not revealed based on AMOVA analysis as only 5% variance was observed among temporal groups compared to 95% within group variation. Similar results were obtained in European barley cultivars, wherein only 2.7% variance was due to differences between different temporal groups[22]. But compared to temporal diversity, spatial groups based on developing centers of the varieties as well as their indigenous and exogenous origin accounted for 15% and 14% between group variations respectively, which is also reflected in the resulting spatial groups (GI and GII) obtained based on Structure analysis with some exceptions. Structure data revealed spatial distribution based on their exotic and indigenous origin as well as place of developing/releasing varieties. It also revealed grouping of malt, two-rowed and hulless varieties in one group and six-rowed, hulled and feed barley of indigenous origin in another group with some exception. The results suggested the uniqueness of these varieties for placing them in different sub-clusters/groups based on Structure and UPGMA cluster analysis.

Conclusion

The present study revealed the trend of genetic diversity over the decades; the spatial diversity available in barley varieties released from different centers in India and also with respect to the usage of exotic and indigenous germplasm in varietal development. The barley varietal release system resulted in significant increase in genetic diversity over the initial three decades and mild increase in last two decades. Hence, there is a need to use more diverse germplasm to further strengthen the genetic base of barley varieties., Further, spatio-temporal studies on varieties released from time to time may help crop improvement.

Conflict of Interest

The authors declare that there is no conflict of interests.

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