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# Evaluating Genetic variation in barley varieties at molecular level

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***Abstract:*** *Information on genetic diversity and relationship among genotypes is a vital part in order to capitalize the benefit for achieving transgressive segregants in self pollinated crops like barley. Forty nine barley varieties representing different growing zones were analyzed for genetic variability using PIC statistics and genetic similarity based clustering. A set of 36 SSR markers were used for the study, out of which 24 were found polymorphic. In total 53 alleles were scored in selected genotypes and 1769 data points were generated for PCR based amplification profiles. The number of alleles ranged from 1 to 3 with an average of 1.76 alleles per locus. The band fragment size varied from 100 bp to 600 bp with PIC values ranging from 0.0 to 0.73. Allele molecular weight data of amplified profiles were converted to develop binary format (allele presence = "1" and allele absence = "0") for diversity analysis using DARwin software package. The dendrogram generated grouped Indian barley varieties in four groups depending upon their genetic variability. The eventual intend of this study is to develop molecular markers based amplification profiles of Indian barley cultivars for varietal characterization and molecular variability assessment.*

***Keywords:*** *Barley, genetic variability, SSR markers, hierarchical clustering*

## INTRODUCTION

Barley (*Hordeum vulgare L.*) is the fourth leading cereal crop in the world, after rice, wheat, and corn, based on area and production globally. IT is one of the ancient crop species in the world and has been subjected to considerable genetic variability. Domestication of wild barley in various geographical areas could explain the highly diverse forms of barley, which are subjected, for a long time, to a new environmental pressures and leads to development of geographical races. This tangential genetic diversity of related wild species or crop ancestors is important to solve problems related to crop failure due to environmental and biological stresses. Therefore, development of highly productive new cultivars with good quality properties over a long period of time requires use of these highly diverse germplasm to increase the chances for success in breeding programme (Malysheva-Otto *et al.*, 2006).

In recent years, the demand for barley based natural, functional, and healthy foods has tremendously increased that resulted in development and release of mosaic of barley cultivars (Kumar *et al.*, 2014).Variability among these cultivars is mostly determined via agronomic and biochemical characters, which are, however strongly influenced by the environment (Russel *et al.*, 1997). Therefore, molecular based identification has become increasingly important these days under the growing number of varieties, for proper documentation of genetic resources and for the protection of the breeders' interests. Molecular markers detect differences directly at the DNA level and are not influenced by the environment. However, the extent of their utility in a crop species may depend on the nature of the markers, their number, genome coverage and the population under investigation as well as their linkage to traits of interest (Davila *et al.*, 1999; Pillen *et al*. 2000).

The advent of the Polymerase Chain Reaction (PCR) favored the development of different molecular techniques such as Random Amplified of Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR or microsatellite), Sequence Tagged Sites (STS), Random Amplified Microsatellite Polymorphism (RAMP), Single Nucleotide Polymorphisms (SNPs)

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and Diversity Array Technology (DArt) (Davila *et al.*, 1999; Malysheva-Otto *et al.*, 2006; Wenzl *et al.*, 2006) Molecular markers have extensively used in varietal characterization and evaluation of genetic diversity within them in many crops. Various studies were conducted using arrays of marker technologies for detecting genetic diversity, genotype identification and genetic mapping in barley. Still microsatellite or simple sequence repeats (SSRs) based marker technology is most suited for marker-assisted selection (MAS) and genetic diversity studies (Wei *et al.*, 2015). The present study is also conducted to develop SSR markers based amplification profiles of Indian barley cultivars for varietal characterization and genetic variability purposes.

## MATERIAL AND METHODS

### Plant materials and DNA isolation

A set of 49 barley varieties released under All India Coordinated Wheat and Barley Improvement Project (AICW&BIP) were used (Table 1) for the current study. Seed material of these varieties was procured from Barley Section of Indian Institute of Wheat & Barley Research, Karnal.

### DNA extraction

Equal number of fresh, young leaves (two weeks old seedlings) of at least six plants from each genotype was bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai Maroof *et al.*, 1984). The DNA samples were analyzed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis.

### Generation of SSR markers based molecular profiles

Total 36 SSR markers were selected from different locations of each linkage group of barley genome as given in Table 2. The sequence of these primer pairs, their Tm and amplified fragment and PCR conditions were obtained from website *(www.wheat.pw.usda.gov/cgi-bin/graingenes.com).* PCR reaction was conducted in a reaction volume of 20 µl containing 1X PCR buffer. 200 m M dNTPs, 0.25 µM of primer, 2Mm MgCl2, 1u 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-bpDNA ladder (NEB, UK) and corroborated with the

**Table 1**
**Salient features of Indian barley varieties used in this study**

| *Variety* | *2/6 row* | *Hulled/ naked* | *Growing zone* | *Production conditions* |
|---|---|---|---|---|
| Alfa 93 | 2 | H | NWPZ | IRTS |
| Azad | 6 | H | NEPZ | IRTS |
| BCU 73 | 2 | H | NWPZ | IRTS |
| BH393 | 6 | H | NWPZ | IRTS |
| BH885 | 2 | H | HARYANA | IRTS |
| BH902 | 6 | H | NWPZ | IRTS |
| BHS352 | 6 | N | NHZ | RFTS |
| BHS 380 | 6 | H | NHZ | RFTS |
| BHS 400 | 6 | H | NHZ | RFTS |
| BHS 46 | 6 | H | NHZ | RFTS |
| Clipper | 2 | H | NWPZ | IRTS |
| DL 88 | 6 | H | NWPZ, PZ | IRLS |
| Dolma | 6 | N | NHZ | RFTS |
| DWR 28 | 2 | H | NWPZ | IRTS |
| DWRUB 52 | 2 | H | NWPZ | IRTS |
| DWRUB 64 | 6 | H | NWPZ | IRLS |
| DWRB73 | 2 | H | NWPZ | IRLS |
| DWRB 91 | 2 | H | NWPZ | IRLS |
| DWRB 92 | 2 | H | NWPZ | IRTS |
| DWRB 101 | 2 | H | NWPZ | IRTS |
| Gitanjali | 6 | N | UP | RFTS |
| HBL 276 | 6 | N | NHZ | RFTS |
| HUB 113 | 2 | H | NEPZ | IRTS |
| JB 58 | 6 | H | MP | RFTS |
| Jyoti | 6 | H | NEPZ | IRTS |
| K 141 | 6 | H | NEPZ | RFTS |
| K 508 | 6 | H | NEPZ | IRTS |
| K 551 | 6 | H | NEPZ | IRTS |
| K 560 | 6 | H | NEPZ | RFTS |
| K 603 | 6 | H | NEPZ | RFTS |
| Karan 16 | 6 | N | NWPZ | IRTS |
| NB 2 | 6 | H | SAL/ALK | IRTS |
| NDB 1173 | 6 | H | SAL/ALK | IRTS |
| NDB 943 | 6 | N | UP | IRTS |
| PL 419 | 6 | H | Punjab | RFTS |
| PL 426 | 6 | H | PUNJAB | IRTS |
| PL 751 | 6 | H | CZ | IRTS |
| RD 2035 | 6 | H | NWPZ | IRTS |
| RD 2052 | 6 | H | NWPZ | IRTS |
| RD 2508 | 6 | H | NWPZ | RFTS |
| RD 2552 | 6 | H | NWPZ | IRTS |
| RD 2624 | 6 | H | NWPZ | RFTS |
| RD 2660 | 6 | H | NWPZ | RFTS |
| RD 2668 | 2 | H | NWPZ | IRTS |
| RD 2715 | 6 | H | CZ | IRTS |
| RD 2786 | 6 | H | SAL/ALK | IRTS |
| RD 2794 | 6 | H | NWPZ | IRTS |
| UPB 1008 | 2 | H | NHZ | RFTS |
| VLB 118 | 6 | H | NHZ | RFTS |

H=hulled, N=naked, IR=irrigated, RF=rainfed, TS=timely sown, LS=late sown, NWPZ=North western plains zone, NEPS= North western plains zone, CZ= Central zone, SAL/ALk= saline-alkaline soils of NWPZ & NEPZ, spike type 2/6= two or six row type spik

reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by re-amplification under similar PCR conditions. Gels were stained with ethidium bromide (0.5ug/ml) and DNA banding patterns were visualized under UV light (Syngene Synoptics Ltd. USA).

**Molecular Data analysis**

Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus, major allele frequency and frequency of major alleles were determined. Polymorphic information content (PIC) was calculated for each SSR marker vides estimates of the discriminatory power of locus by taking into account the number of alleles that are expressed (Anderson *et al.*, 1993).PIC value were calculated as

$$PIC = 1- \Sigma P^2_{ij}$$

Where, $P^2_{ij}$ is the frequency of the $i^{th}$ allele.

Allele molecular weight data were also used to export the data in binary format (allele presence = "1" and allele absence = "0") and entered into a matrix. Based on the matrix of (GD) values, the DARwin software was used to obtain the Hierarchical Clustering, depicting genetic relatedness of the cultivars.

## RESULTS AND DISCUSSION

**SSR markers based molecular polymorphism**

A set of 36 SSR markers were used to characterize 49 Indian barley cultivars at molecular level to assess genetic variability. Out of 36 markers only 22 primer pairs showed polymorphism in selected cultivars as shown in table 2. Total 46 alleles were amplified with an average of 2.09 alleles per locus. Polymorphism information ranged from 0.273 to 0.751 with an average of 0.445 for 22 polymorphic loci. Band fragment size ranged from 100 to 250 bp in this study. Maximun three alleles were observed for SSR markers Bmag 709 (1H) and Bamag 603 (3H). Maximum PIC was observed for Bmag 603 (0.751) whereas minimum was observed for Bmac 224 (0.273). Linkage group 3H with an average PIC value 0.594 appeared to be most variable among Indian barley varieties followed by Linkage groups 2H (0.555) and 1H (.526), respectively. Linkage group 6H was observed least diverse among 49 genotypes with an average PIC value 0.351. Previous reports of SSR markers based variability studies in barley reported average level of polymorphism during genotypic characterization at molecular level (Wei *et al.*, 2015; Jaiswal *et al.*, 2010; Matus and Hayes, 2002; Pillen *et al*, 2000). In present

**Table 2**
**Allelic variation of the polymorphic SSR loci in barley genotypes on the basis of allele richness and PIC**

| *Sr No* | *Molecular marker* | *Chr* | *Tm ($^0$C)* | *No. of alleles* | *Allele size range (bp)* | *PIC* |
|---|---|---|---|---|---|---|
| 1. | Bmac 213 | 1H | 58 | 2 | 140-168 | 0.526 |
| 2. | Bmag749 | 2H | 55 | 3 | 150-166 | 0.636 |
| 3. | EBmatc39 | 2H | 58 | 2 | 140-190 | 0.474 |
| 4. | Bmac129 | 3H | 58 | 2 | 130-189 | 0.438 |
| 5. | Bmag603 | 3H | 55 | 3 | 100-120 | 0.751 |
| 6. | ABG500 | 4H | 58 | 2 | 120-145 | 0.506 |
| 7. | Bmac163 | 5H | 55 | 2 | 130-146 | 0.476 |
| 8. | Bmag222 | 5H | 58 | 2 | 155-179 | 0.494 |
| 9. | Bmag223 | 5H | 58 | 2 | 127-180 | 0.338 |
| 10. | Bmag760 | 5H | 55 | 2 | 100-110 | 0.456 |
| 11. | Bmag812 | 5H | 55 | 2 | 140-157 | 0.408 |
| 12. | Bmac40 | 6H | 58 | 2 | 110-145 | 0.300 |
| 13. | GBM1215 | 6H | 60 | 2 | 200-250 | 0.316 |
| 14. | HVM11 | 6H | 55 | 2 | 150-181 | 0.439 |
| 15. | Bmac64 | 7H | 58 | 2 | 140-155 | 0.415 |
| 16. | Bmac167 | 7H | 55 | 2 | 150-176 | 0.452 |
| 17. | Bmac224 | 7H | 55 | 2 | 150-166 | 0.273 |
| 18. | Bmac273 | 7H | 55 | 2 | 110-186 | 0.531 |
| 19. | Bmac297 | 7H | 55 | 2 | 180-206 | 0.494 |
| 20. | Bmac579 | 7H | 55 | 2 | 100-126 | 0.378 |
| 21. | Bmag110 | 7H | 58 | 2 | 123-150 | 0.316 |
| 22. | Bmag341 | 7H | 55 | 2 | 200-215 | 0.382 |

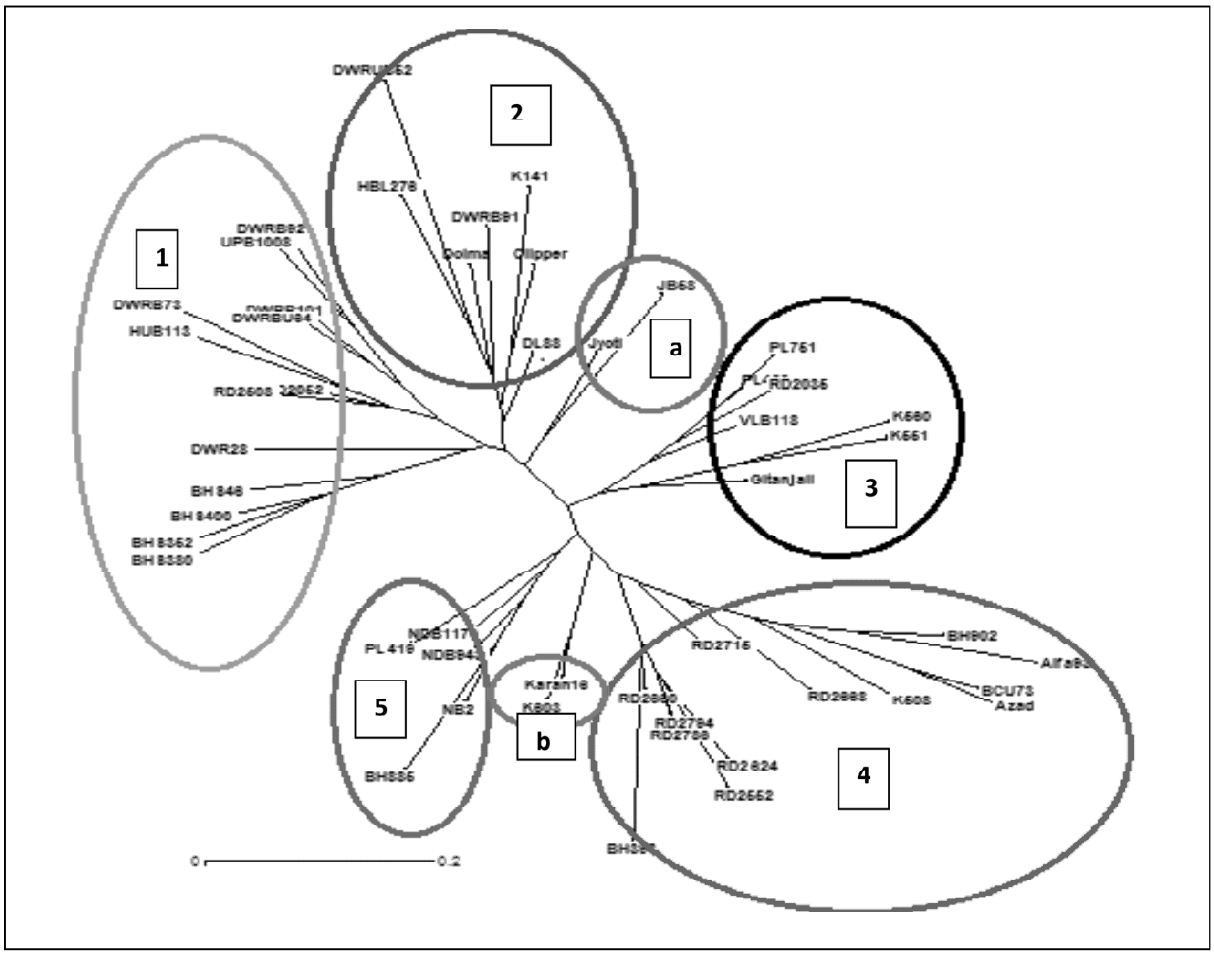


**Figure 1: Hierarchical clustering of Indian barley varieties obtained on the basis of SSR markers**

study, most of markers produced only few alleles and despite their ability to produce only few alleles, they were robust enough to distinguish different accessions of the same genotype as reported in previous studies (Russel *et al.*, 1997; Jaiswal *et al.*, 2010). This average variability observed for barley varieties is also attributed towards the biased selection of parental lines during varietal breeding programme to reap maximum for yield gains that mostly results in narrowed genetic diversity in most of cereal crops.

**Molecular polymorphism based hierarchical clustering of barley varieties**

In order to examine genetic relationships among the 49 barley genotypes based on the SSR markers variability, the data scored from the 22 primer pairs were compiled and analyzed using DARwin software programme to obtain the hierarchical clustering depicting genetic relatedness of the cultivars. As shown in figure 1. Selected 49 cultivars were clustered into five major groups and two minor groups. First cluster grouped the varieties like DWRB73, DWRB92, DWRUB64 and UPB1008 which were derived from the indigenous parental lines. On contrary, second cluster contained the varieties including Clipper, Dolma, HBL278 and K141 that were developed from the crosses between Indian and exotic parental combinations. Third group clustered the feed barley varieties released for central India whereas fourth clustercombined irrigated varieties of north-west region of India. The fifth group clubbed varieties which are salinity tolerant and developed for north east barley sowing region. The minor groups grouped two varieties each as irrigated varieties of north-eastern and central regions of India in group (a) and one hulled and one naked variety in group (b), respectively. Similar clustering was observed in previous studies reported for molecular markers based clustering for genetic variability in barley (Malysheva-Otto *et al.*, 2006; Wei *et al.*, 2015). The distribution of our studied barley varieties in different clusters showed that either each group obtained in

this study share the same agronomic character or varieties were clustered according to their same eco-geographical region. This clustering of barley cultivars thus exhibited similar genetic relatedness within each cluster and variability among different clusters as reported previously by Malysheva-Otto *et al*. (2006). This relative relationship observed between SSR markers and the geographic origin of the Indian barley accessions maybe explained by the long term adaptive conditions under the specific regions or sub-regions of each country. These particular conditions may influence the cultivar behavior and lead to some traits of adaptation such as earliness to avoid water deficitor small spike that will be rapidly filed and so on. However,it is suggested that more molecular data is required to distinguish accessions coming from the same region and consequently more efficient utilization of existing variability for improvement of barley in India.

## CONCLUSION

This study concludes that there is an average genetic variability in Indian barley varieties as observed by SSR based molecular markers. This present finding strengthens previous reports on the correlation between eco-geographical distribution and SSR markers. It shows also that molecular markers can be used effectively to estimate genetic distances among genotypes and distinguish between naked and hulled barley accessions. The usefulness of polymorphic SSRs identified in this study mar be used for assessing genetic diversity, cultivar identification and understanding genetic variation within populations in future for the efficient use of genetic resources in a breeding program and plant varietal protection.

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