# Diversity in Himalayan Hull-less Barley (*Hordeum vulgare* L.) Landraces Using AFLP and STMS Markers

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A total of 28 accessions naked barley landraces mainly from north-western Indian Himalaya comprising three main administrative regions, Jammu & Kashmir, Himachal Pradesh and Uttarakhand states together with five exotic accessions, four from Ethiopia and one from Nepal, were characterized using AFLP and STMS markers. The AFLP markers resolved inter-population diversity better particularly discriminating accessions from Uttarakhand Himalaya with specific adaptations. STMS markers used for studying population genetic parameters revealed that accessions from Jammu & Kashmir and Himachal Pradesh were more diverse than accessions from Uttarakhand and the exotic accessions. The group-wise F-statistics revealed that on average 68% of variation was apportioned among populations and 32% within populations. Understanding the population genetic parameters for *in situ* (on-farm) management of farmers' landraces and use of landraces representing locally common alleles for specific adaptations in crop improvement is suggested.

**Key words: Naked barley, *Hordeum vulgare* L., Genetic diversity, North-western Himalaya, Molecular characterisation**

Cultivated barley (*Hordeum vulgare* ssp. *vulgare*) can be classified into covered (hulled), and naked (hull-less) forms. Most barley varieties grown presently are of the hulled form, and they are mainly used for brewing malt and animal feed. By contrast, naked barley is produced on a small scale and used mainly as human food because of the ease in processing and edibility. Naked barley is attracting attention in recent years, as feed and as a healthy food because of the high feed value and abundance of dietary fibre, respectively (Liu *et al.*, 1996). According to a survey by Takahashi (1955), naked barley is distributed widely, but its frequency greatly differs among regions; naked barley accounts for more than 95% of the domesticated barley in the highlands of Nepal and Tibet, and almost 50% in China, Korea and Japan, but the frequency decreases toward the west, becoming low in Europe. In the regions where naked barley is grown at high frequencies, it is an important staple food.

The Himalayan barleys, particularly naked forms, are of great potential utility, which require research and marketing interventions. The Himalayas, Ethiopia, and Morocco have all been considered as centres of barley domestication (Åberg, 1938; Bekele, 1983; Molina-Cano *et al.*, 1987). The widespread distribution of naked barley makes the hulled or naked caryopsis character a key trait to follow the origin and domestication process of barley (Harlan, 1995; Salamini *et al.*, 2002). A recent study (Taketa *et al.*, 2004), however, indicates that naked barley has a monophyletic origin, probably in southwestern Iran, and all naked barleys are likely to share a common ancestor.

In many areas in North-western Himalaya in proximity of Tibet, naked barley was widely cultivated till about 4 or 5 decades ago but its cultivation has gone down considerably in recent years. Naked barley landraces have gradually been replaced by wheat in these areas as a cereal of direct human consumption.

About 400 barley landraces including naked types have been collected recently from north-western Himalaya during 1999-2004 and are being maintained *ex situ* in National Genebank at the National Bureau of Plant Genetic Resources (NBPGR). These landraces are yet to be systematically characterized/evaluated. Limited information is, therefore, available on genetic diversity of existing naked barley landraces grown in north-western Himalayas. The extent of diversity between populations adapted to the high altitude region of north-western Himalayas would suggest need for their continuous collection and devising strategies for both *ex situ* and *in situ* (on-farm) conservation. It would also suggest possibility of utilizing the useful genetic variation in these landraces for improved yield and a better use of these products in agriculture and industry. Morphological diversity in naked barley landraces collected from north-western Himalaya was studied (Venkatesan *et al.* 2005). In the present paper, molecular diversity in selected barley populations from north-western Himalaya was studied using Amplified Fragment Length Polymorphism (AFLP) and Sequence Tagged Microsatellite Sites (STMS)

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markers. While AFLP markers were used for inter-population diversity, the STMS markers were used mainly for study of population genetic parameters (intra-population variation).

## Materials and Methods

The experimental material consisted of 28 naked barley populations assembled from different parts of north-western Himalayas and represented all agro-ecological conditions of the region. The material also included 5 naked barley landraces from exotic sources including 4 from Ethiopia and one from Nepal. The passport information on the origin of accessions is given in Table 1. Procedures involved in AFLP and STMS analyses are detailed below:

### *DNA Extraction*

Extraction of total genomic DNA was carried out using method given by Saghai-Maroof *et al.* (1984) with minor modification. In each accession, healthy young leaves of same size were taken from 30 plants (6 weeks old) for bulk DNA extraction for AFLP characterization. For intra-population diversity analysis using STMS markers, DNA was extracted from 10 individual plants from each barley population.

**Table 1. List of selected barley accessions for detailed diversity analysis**

| S.No. | Accession | Origin |
|---|---|---|
| 1. | IC 260864 | Uttarakhand |
| 2. | IC 266944 | Uttarakhand |
| 3. | IC 355879 | Uttarakhand |
| 4. | IC 356114 | Uttarakhand |
| 5. | IC 356122 | Uttarakhand |
| 6. | IC 362237 | Uttarakhand |
| 7. | IC 362256 | Uttarakhand |
| 8. | IC 398291 | Uttarakhand |
| 9. | IC 406567 | Uttarakhand |
| 10. | IC 406568 | Uttarakhand |
| 11. | IC 406569 | Uttarakhand |
| 12. | IC 406570 | Uttarakhand |
| 13. | IC 406571 | Uttarakhand |
| 14. | IC 406572 | Uttarakhand |
| 15. | PI 176040 | Uttarakhand |
| 16. | KC/PS 37 | Uttarakhand |
| 17. | PI 429907 | Jammu & Kashmir |
| 18. | PI 429885 | Jammu & Kashmir |
| 19. | PI 429895 | Jammu & Kashmir |
| 20. | PI 429889 | Jammu & Kashmir |
| 21. | PI 429896 | Jammu & Kashmir |
| 22. | PI 429941 | Himachal Pradesh |
| 23. | PI 572605 | Himachal Pradesh |
| 24. | CIHO 3228 | Nepal |
| 25. | PI 316815 | Ethiopia |
| 26. | PI 356131 | Ethiopia |
| 27. | PI 382537 | Ethiopia |
| 28. | PI 382556 | Ethiopia |

### *AFLP Analysis*

Plant mapping kit for regular plant genomes (500-6000 Mb) AFLP™ (PE Applied Biosystems, USA) was used for the analysis. The technique developed by Vos *et al.* (1995), was optimized for barley by modifying the protocol provided by the manufacturers. The technique is based on the selective PCR amplification of restriction fragments from digestion of total genomic DNA. ABI plant mapping protocol was followed for analysis of barley populations. The recommended DNA concentration was 500 ng per reaction. The DNA concentration was optimized for barley by testing three different DNA concentrations *viz.*, 500, 200 and 100 ng per reaction. Similarly, two restriction enzyme concentrations *viz.*, 5U and 3U of *EcoR* I per reaction and 1U and 0.2U of *Mse* I were tested and finally 3U of *EcoR* I and 0.2U *Mse* I enzymes were used. Pre-selective amplification was performed to reduce the overall complexity of the target DNA fragments by increasing specific target sequences so that the double digested fragments become predominant. This was performed by using pre-selective primers (Table 2).

Each peak in the electropherogram was considered as an AFLP marker. The scoring of bands was done from the electropherograms according to size (in bp.). Presence of band was treated as '1' and absence of band as '0'. The 'Genescan' and 'Genotyper' software packages (Applied Biosystems, USA) were used for processing and scoring of the results. The 1-0 matrix of cultivars X AFLP products was used to calculate pairwise Jaccard's similarity coefficient.

The similarity coefficient matrix was subjected to cluster analysis by Unweighted Pair Group Method of Arithmetic Average (UPGMA) analysis and dendrogram was generated with the help of NTSYS-pc software version 1.70 (Exeter Software, New York, USA). The 28 selected barley landraces were used in the cluster analysis.

### Microsatellite (STMS) Analysis

The procedure described below was used for carrying out the PCR reactions for STMS analysis. The PCR reaction mixture consisted of Taq DNA polymerase, 10x PCR buffer, dNTPs, $MgCl_2$, primer and genomic DNA. Optimization of PCR component concentrations was carried out for Taq DNA polymerase, $MgCl_2$, genomic DNA and primer. Concentration of dNTPs (200 iM) and PCR buffer (1x) was not varied. PCR reactions were carried out in a Perkin Elmer Thermocycler with heated

lid. Thermocycling conditions were as follows: 1) denaturation at 94° C for 3 minutes; 2) 45 cycles of denaturation at 94° C for 1 minute, 3) primer annealing at 55° C for 1 minute; 4) primer extension at 72° C for 2 minutes and 5) final extension step at 72° C for 10 minutes. The selected primers after screening and their sequences are listed in Table 3. The selected five primers were used for population genetic studies.

Electrophoresis was carried out at 90 V for one hour 30 minutes till the bromophenol blue dye travelled less than 2/3rd the length of gel. The resolved amplification products were visualized under UV light after staining with ethidium bromide on a UV-Transilluminator. The gel was photographed using a Gel Documentation System.

The amplification products were scored across the lanes comparing their respective molecular weights. Each band was treated as one STMS allele. Scoring of bands was done from photographs. Homology of bands was based on distance of migration in the gel.

The STMS allele data for each population was used to calculate frequency of occurrence of a specific allele. The accessions x allele matrix for all 5 loci analysed was used to calculate the genetic diversity parameters. Genetic diversity of the populations was described using the parameters, per cent polymorphic loci, number of alleles per locus, genetic diversity for each locus and population, the level of heterozygosity, coefficient of gene diversity, Shannon diversity index, fixation-indices and gene flow for all loci.

## Results

### *AFLP Analysis*

#### *Primer Screening*

Twelve selected primer pairs were used for detailed genetic diversity analysis for 28 barley landraces. They were selected from the list published in literature. The properties of AFLP amplicons generated by 12 primers in selected barley landraces are listed in Table 2. The selected primer pairs generated a total of 1517 amplicons ranging from 36 (E-ACC/M-CAC) to 188 (E-ACA/M-CTT) among 28 barley landraces analyzed. Of the 1,517 amplicons generated 1,318 amplicons (86.9%) were polymorphic and ranged from 34 (E-ACC/M-CAC) to 159 (E-ACG/M-CAA). The per cent polymorphism ranged from 81.1% to 98.0%. Among twelve primer pairs, four primer pairs generated above 90% polymorphism (E-ACC/M-CAA, E-ACC/M-CAC, E-ACG/M-CAA and

**Table 2. Properties of AFLP amplicons generated by 12 primers in selected barley landraces**

| S.No. | Primer sequence | Total no. of amplicons | No. polymorphic amplicons | % polymorphism | Average no. of amplicons per accession |
|---|---|---|---|---|---|
| 1 | E-ACA/M-CAA | 176 | 153 | 86.9 | 93.4 |
| 2 | E-ACG/M-CAA | 172 | 159 | 92.4 | 80.7 |
| 3 | E-ACC/M-CAA | 51 | 50 | 98.0 | 11.2 |
| 4 | E-ACA/M-CAC | 157 | 143 | 91.1 | 68.4 |
| 5 | E-ACG/M-CAC | 127 | 114 | 89.8 | 58.0 |
| 6 | E-ACC/M-CAC | 36 | 34 | 94.4 | 8.6 |
| 7 | E-ACA/M-CTG | 127 | 106 | 83.5 | 68.3 |
| 8 | E-AAG/M-CTG | 102 | 89 | 87.3 | 47.8 |
| 9 | E-ACC/M-CTG | 127 | 103 | 81.1 | 61.8 |
| 10 | E-ACA/M-CTT | 188 | 153 | 81.4 | 99.6 |
| 11 | E-AAG/M-CTT | 127 | 107 | 84.3 | 59.8 |
| 12 | E-ACC/M-CTT | 127 | 107 | 84.3 | 55.0 |
| | Total | 1517 | 1318 | 86.9 | 712.6 |

**Table 3. Primer sequences, repeat motif, allele size ranges (bp), and chromosomal location of 5 microsatellite primers**

| Primer ID | Primer sequence (5'–3') | Core motif | Allele size | Annealing temperature | Chromosomal location* |
|---|---|---|---|---|---|
| HVBKAS1 | attggcgtgaccgatatttatgttca caaaactgcagctaagcaggggaaca | (CA)n | 176-204 | 56 | 2(2H) |
| HVM9 | cttcgacaccatcacccag accaaaatcgccatcgaacat | $(TCT)_5$ | 218-230 | 54 | 3(3H) |
| HVM43 | ggattttctcaagaacactt gcgtgagtgcataacatt | $(CA)_9$ | 239 | 50 | 5(1H) |
| BMS90 | acatcaaccctcctgctc ccgcacatagtggttacatc | $(AC)_{20}$ | 221 | 55 | 5(1H) |
| HVM6 | catggatgaatgattggtttg cgcgtccgtatgtgtatgagtaa | $(GA)_9$ | 175 | 52 | 7(5H) |

* Chromosome numbering follows barley nomenclature with the homologous chromosome group given in parenthesis (IBGS, 1996)

E-ACA/M-CAC). The average number of amplicons per accession ranged from 8.6 (E-ACC/M-CAC) to 99.6 (E-ACA/M-CTT) per primer. Of the 12 primer pairs, two primer pairs showed the average number of amplicons per accession as above ninety. AFLP profiles of two primer pairs are presented in figure 1.

### Diversity Analysis

The Jaccard's similarity coefficients ranged from 0.37 to 0.72. The UPGMA dendogram (Fig. 2) constructed from Jaccard's similarity co-efficients indicated presence of two major clusters, when cut-off value was considered as 0.546, the average similarity coefficient. The cluster I included 23 barley populations representing all regions, Uttarakhand, Jammu & Kashmir, Himachal Pradesh, Nepal and Ethiopia. The cluster could be further divided into two sub-clusters, sub-cluster Ia comprising only two accessions from Uttarakhand (IC-266944 and IC-406570). The cluster II comprised only two populations, IC-356122 and IC-406567, both from Uttarakhand. The Jaccard's similarity co-efficient between these accessions is 0.53. The three accessions, from Uttarakhand (IC- 260864 and IC-406572) and one accession from Ethiopia (PI- 382556) were not included in any of the clusters.

### Microsatellite (STMS) Analysis

#### *Primer Screening*

Five primers were used for detailed population genetic diversity analysis for 28 barley landraces. These primers were selected from the list published in literature. The properties of STMS bands generated by five primers are listed in Table 3. This Table included the core motif, allele size etc. The Alleles had simple core repeat motifs *viz.*, $(C)_n$, $(TCT)_n$, $(GA)_n$, $(CA)_n$ except two, $(AG)_{11}.(GA)_{14}$ and $(GA)_6.(GT)_4.(GA)_7$.

The Allele frequency per locus generated by five STMS primers among populations is listed in Table 4. The primer HVBKASI identified two alleles per locus with average allelic frequency of 0.21 and 0.79. The primer BMS90 identified two alleles per locus with average allelic frequency of 0.38 and 0.62. The primer HVM43 identified three alleles with average allelic frequency of 0.028, 0.967 and 0.003. The primers HVM9 and HVM6 identified only single allele with allelic frequency of 1.00. A representative profile generated by two STMS primers is presented in figure 3.

### Diversity Analysis of STMS Data

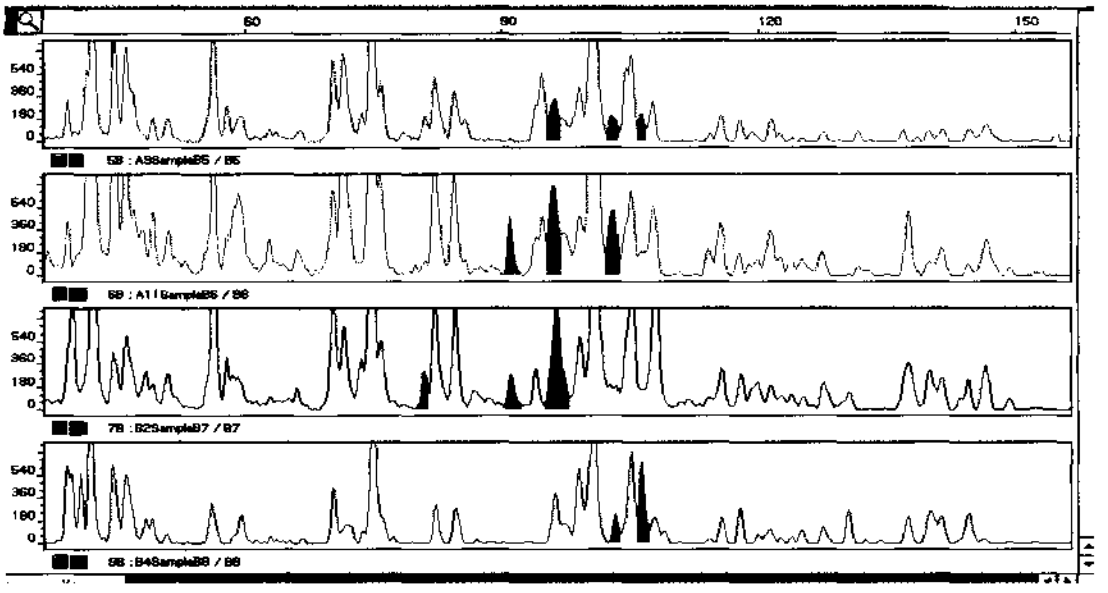
The summary statistics of diversity analysis of selected barley landrace populations is listed in Table 5. Of the five loci analysed by selected primers, three loci were polymorphic and two loci were monomorphic. The percentage of polymorphic loci is 60%. Of the observed number of alleles (1.8±0.82) among populations, the effective number of alleles is 1.25 ± 0.34. The populations with more effective number of alleles possessed higher diversity than the others. These populations were CHIO 3228 (Nepal), PI 572605 (Himachal Pradesh), PI 429885 (Jammu & Kashmir), PI 316815 (Ethiopia), IC 398291 (Uttarakhand), IC 356114 (Uttarakhand), IC 260864 (Uttarakhand) and PI 429941 (Himachal Pradesh). The expected heterozygosity was 0.16±0.20 among populations and the observed heterozygosity was 0.01 ± 0.20. Of the 28 barley populations, only four populations namely, PI-429885 (Jammu & Kashmir), PI-176040 (Uttarakhand), PI-316815 (Ethiopia) and PI-356131 (Ethiopia) showed higher observed heterozygosity.

Shannon's information index was high for populations CHIO 3228 from Nepal, PI 572605 and PI 429941 from Himachal Pradesh, PI 429885 from Jammu & Kashmir, and IC 260864 and IC 362256 from Uttarakhand indicating greater diversity of these populations.

The UPGMA dendrogram from population analysis (Fig. 4) constructed from Nei's genetic distances indicated presence of two major clusters I and II. Cluster I could be further divided into two sub-clusters. The sub-cluster Ia included 21 barley populations, representing accessions from all areas, Uttarakhand, Jammu & Kashmir, Himachal Pradesh, Ethiopia and Nepal. The sub-cluster Ib included only three populations, two from Uttarakhand and one from Ethiopia. The cluster II included three populations, all from Jammu & Kashmir. One accession from Uttarakhand (IC-355879) was not included in any of the clusters.

### Grouped Population Descriptive Statistics

The group-wise allelic frequency per locus generated by 5 STMS primers is listed in Table 6. The group I included the accessions collected from Uttarakhand and one accession from adjoining Nepal, in these three loci out of five were polymorphic. The group II included the populations assembled from Jammu & Kashmir in which two loci out of five loci identified by selected primers were polymorphic. The group III comprised the populations from Himachal Pradesh and only two loci out of five loci were polymorphic. The group IV comprised the populations from Ethiopia where only one locus out of five loci was polymorphic.



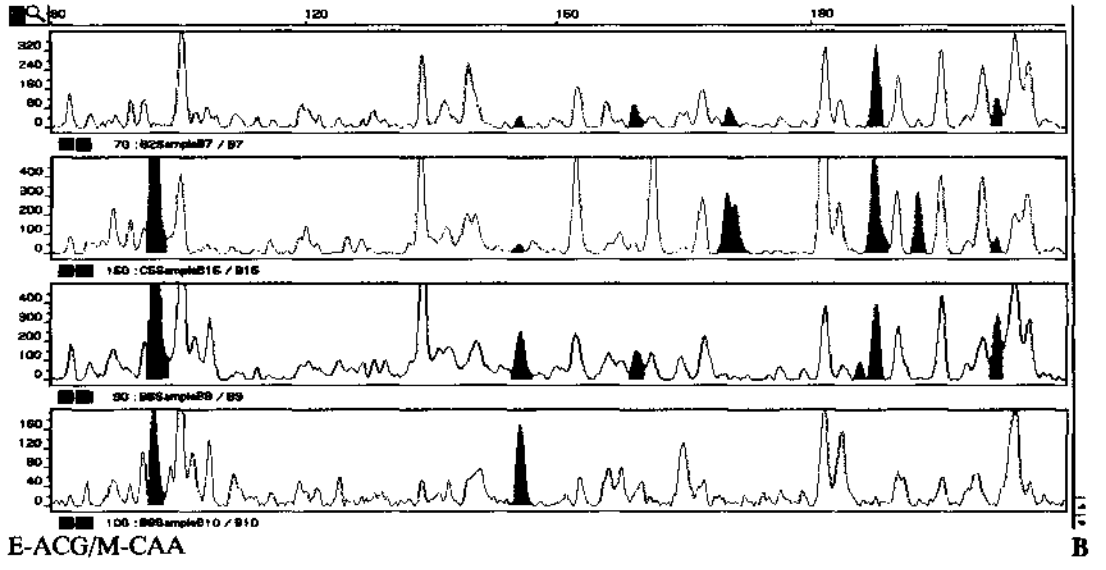




***Fig 1(A,B):*** *AFLP profiles of four barley landraces each generated by different primer pairs indicating the quality and extent of polymorthism for amplification products. The dark coloured filled bands are polymorphic and rest are monomorphic among the populations*

The summary statistics for selected populations group-wise is presented in Table 7. The populations from group I (Uttarakhand and Nepal), group II (Jammu & Kashmir) and group III (Himachal Pradesh) contributed more for representation of diversity of populations with more effective number of alleles. The group I populations had all the three polymorphic loci out of five loci identified by selected primers. The per cent polymorphism of group I population is 60% and both group II and group III is 40%.

The group I, group II and group IV showed higher observed heterozygosity which was possibly the result of natural crossing among the populations. The Shannon's indices were more for group II and group III showing greater diversity of the populations in these groups.

The summary of group-wise F-statistics and gene flow for all loci identified by 5 STMS primers is listed in Table 8. The proportion of diversity among and within populations indicated that, on average 68% (Fst) of the variation was among populations and 32% (1-Fst) was

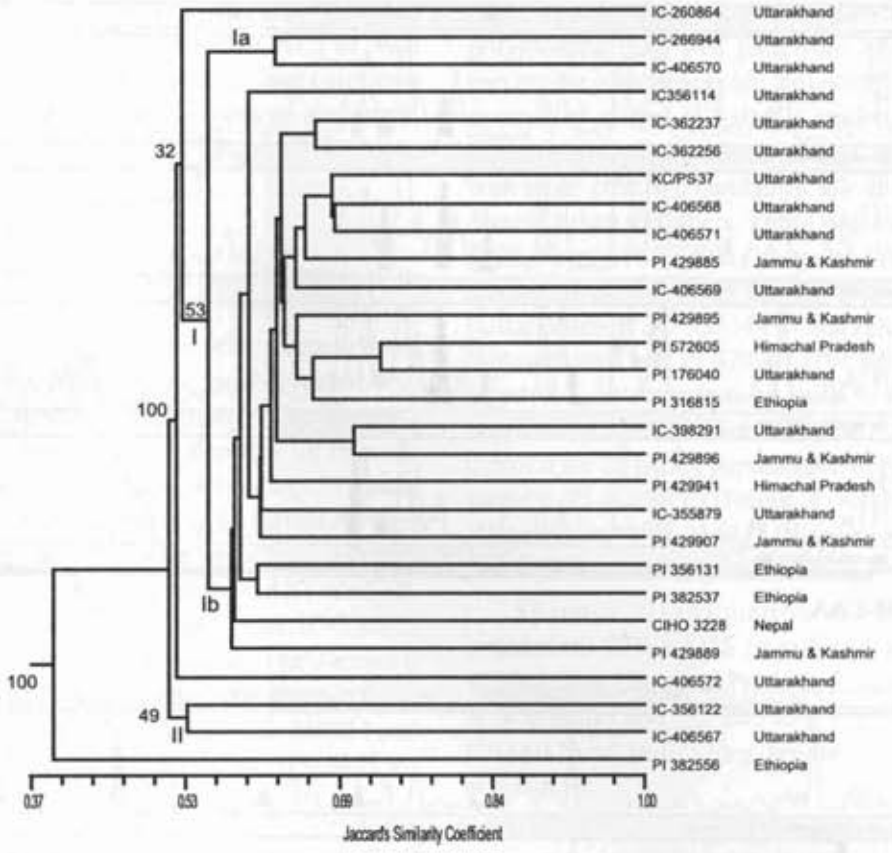



*Fig. 2: UPGMA dendrogram of 28 barley landraces based on AFLP data*

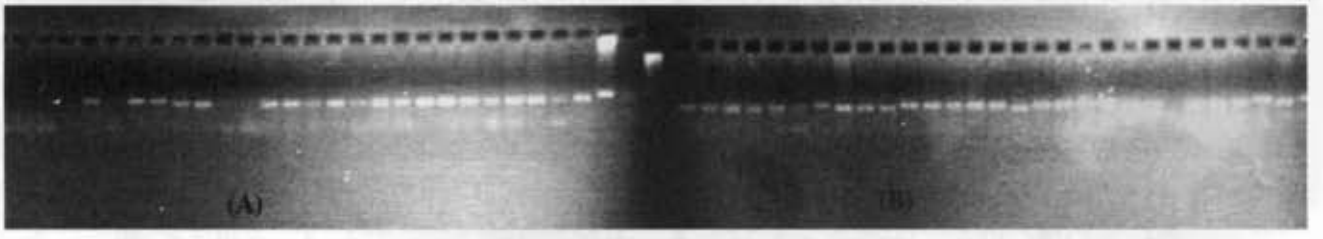



***Fig. 3: STMS profiles of barley populations generated by the primers, HVM 43 (A) and HVBKASI (B) indicating inter-population diversity***

within populations. High Fst values in group II (Jammu & Kashmir) and group I (all populations from Uttarakhand and one accession from Nepal) were indicative of high population differentiation in these groups. The negative Fis values in group II, III and IV were indicative of high degree of inbreeding among populations of these groups. High Fit values in group I, II and III also indicate greater population differentiations between populations.

The contribution of geneflow (Nm values) is more in group III (Himachal Pradesh) populations as compared to other groups.

## Discussion

### *AFLP characterization*

The AFLP characterisation of selected barley landraces indicated enough polymorphism to fully differentiate the inter population diversity (Table 2). The bootstrap tests on the data of figure 2 fully supported the resolution shown. Though, at the major cluster level there was no strong association between geographical origin and genetic diversity, yet at sub-cluster level this association was better revealed. Some of the Uttarakhand accessions were clearly discriminated in distinct clusters/sub-

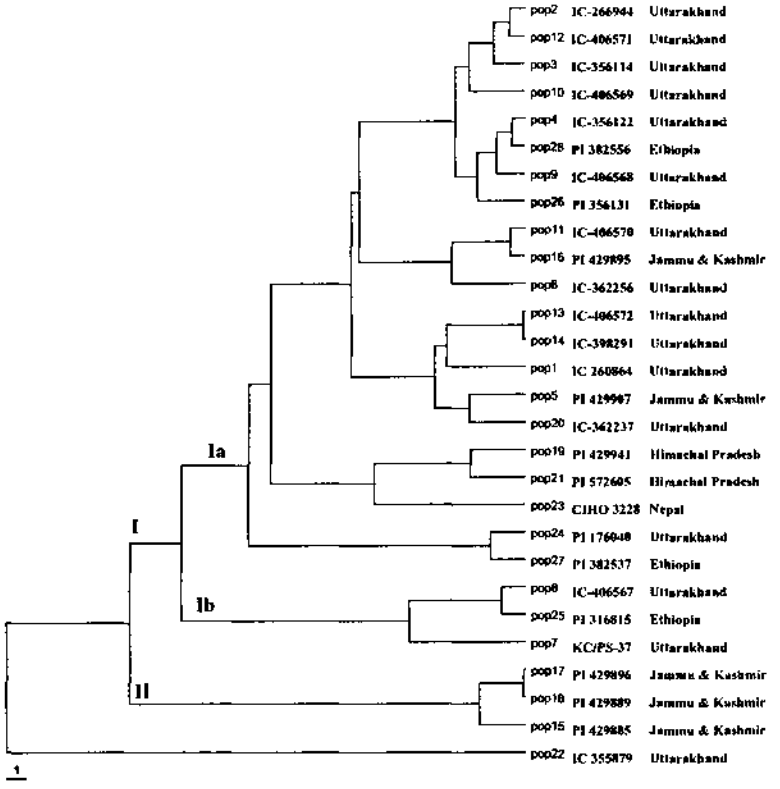



***Fig. 4:*** *UPGMA dendrogram of 28 barley landraces based on STMS data*

**Table 4. Allelic frequency per locus generated by 5 STMS primers**

| S.No. | Accession | HVBKAS1 | | HVM9 | BMS90 | | HVM43 | | | HVM6 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Allele A | Allele B | Allele A | Allele A | Allele B | Allele A | Allele B | Allele C | Allele A |
| 1. | IC-260864 | 0.90 | 0.10 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 2. | IC-266944 | – | 1.00 | 1.00 | 0.10 | 0.90 | – | 1.00 | – | 1.00 |
| 3. | IC-356114 | – | 1.00 | 1.00 | 0.30 | 0.70 | – | 1.00 | – | 1.00 |
| 4. | IC-356122 | – | 1.00 | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 |
| 5. | IC-362237 | – | 1.00 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 6. | IC-362256 | – | 1.00 | 1.00 | 0.90 | 0.10 | – | 0.90 | 0.10 | 1.00 |
| 7. | KC/PS-37 | – | 1.00 | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 |
| 8. | IC-406567 | – | 1.00 | 1.00 | – | 1.00 | 0.10 | 0.90 | – | 1.00 |
| 9. | IC-406568 | – | 1.00 | 1.00 | 0.10 | 0.90 | – | 1.00 | – | 1.00 |
| 10. | IC-406569 | – | 1.00 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 11. | IC-406570 | – | 1.00 | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 12. | IC-406571 | – | 1.00 | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 13. | IC-406572 | – | 1.00 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 14. | IC-398291 | – | 1.00 | 1.00 | 0.70 | 0.30 | – | 1.00 | – | 1.00 |
| 15. | PI 429885 | 0.70 | 0.30 | 1.00 | 0.90 | 0.10 | – | 1.00 | – | 1.00 |
| 16. | PI 429895 | 0.10 | 0.90 | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 17. | PI 429896 | 0.90 | 0.10 | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 18. | PI 429889 | 1.00 | – | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 19. | PI 429907 | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 |
| 20. | PI 429941 | 0.20 | 0.80 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 21. | PI 572605 | 0.50 | 0.50 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 22. | IC-355879 | – | 1.00 | 1.00 | 1.00 | – | – | 1.00 | – | 1.00 |
| 23. | CIHO 3228 | 0.50 | 0.50 | 1.00 | 0.50 | 0.50 | – | 1.00 | – | 1.00 |
| 24. | PI 176040 | – | 1.00 | 1.00 | – | 1.00 | 0.15 | 0.85 | – | 1.00 |
| 25. | PI 316815 | – | 1.00 | 1.00 | – | 1.00 | 0.30 | 0.70 | – | 1.00 |
| 26. | PI 356131 | – | 1.00 | 1.00 | – | 1.00 | 0.25 | 0.75 | – | 1.00 |
| 27. | PI 382537 | – | 1.00 | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 |
| 28. | PI 382556 | – | 1.00 | 1.00 | – | 1.00 | – | 1.00 | – | 1.00 |
| Overall allelic frequency | | 0.21 | 0.79 | 1.00 | 0.38 | 0.62 | 0.028 | 0.967 | 0.003 | 1.00 |

**Table 5. Summary diversity analysis of selected barley landraces**

| S.No. No. | Accession | Sample size | No. of polymorphic loci | % of polymorphic loci | Observed no. of alleles | Effective no. of alleles | Shannon's Information Index | Observed heterozygosity | Expected heterozygosity |
|---|---|---|---|---|---|---|---|---|---|
| 1. | IC-260864 | 10 | 2 | 40 | 1.40±0.55 | 1.14±0.21 | 0.17±0.23 | 0.00 | 0.11±0.15 |
| 2. | IC-266944 | 10 | 1 | 20 | 1.20±0.45 | 1.04±0.10 | 0.07±0.15 | 0.00 | 0.04±0.08 |
| 3. | IC356114 | 10 | 1 | 20 | 1.20±0.45 | 1.14±0.32 | 0.12±0.27 | 0.00 | 0.12±0.27 |
| 4. | IC-356122 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 5. | IC-362237 | 10 | 1 | 20 | 1.20±0.45 | 1.09±0.21 | 0.10±0.22 | 0.00 | 0.07±0.15 |
| 6. | IC-362256 | 10 | 2 | 40 | 1.40±0.55 | 1.09±0.12 | 0.13±0.18 | 0.00 | 0.08±0.10 |
| 7. | KC/PS-37 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 8. | IC-406567 | 10 | 1 | 20 | 1.20±0.45 | 1.04±0.10 | 0.07±0.15 | 0.00 | 0.04±0.08 |
| 9. | IC-406568 | 10 | 1 | 20 | 1.20±0.45 | 1.04±0.10 | 0.07±0.15 | 0.00 | 0.04±0.08 |
| 10. | IC-406569 | 10 | 1 | 20 | 1.20±0.45 | 1.09±0.21 | 0.10±0.22 | 0.00 | 0.07±0.15 |
| 11. | IC-406570 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 12. | IC-406571 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 13. | IC-406572 | 10 | 1 | 20 | 1.20±0.45 | 1.09±0.21 | 0.10±0.22 | 0.00 | 0.07±0.15 |
| 14. | IC-398291 | 10 | 1 | 20 | 1.20±0.45 | 1.14±0.32 | 0.12±0.27 | 0.00 | 0.09±0.19 |
| 15. | PI 429885 | 10 | 2 | 40 | 1.40±0.55 | 1.19±0.31 | 0.19±0.28 | 0.04±0.09 | 0.12±0.19 |
| 16. | PI 429895 | 10 | 1 | 20 | 1.20±0.45 | 1.04±0.09 | 0.07±0.14 | 0.00 | 0.04±0.08 |
| 17. | PI 429896 | 10 | 1 | 20 | 1.20±0.45 | 1.04±0.10 | 0.07±0.15 | 0.00 | 0.04±0.08 |
| 18. | PI 429889 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 19. | PI 429907 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 20. | PI 429941 | 10 | 2 | 40 | 1.40±0.55 | 1.19±0.26 | 0.20±0.27 | 0.00 | 0.13±0.18 |
| 21. | PI 572605 | 10 | 2 | 40 | 1.40±0.55 | 1.29±0.44 | 0.24±0.33 | 0.00 | 0.17±0.25 |
| 22. | IC 355879 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 23. | CIHO 3228 | 10 | 2 | 40 | 1.40±0.55 | 1.40±0.55 | 0.28±0.38 | 0.00 | 0.21±0.29 |
| 24. | PI 176040 | 10 | 1 | 20 | 1.20±0.45 | 1.07±0.15 | 0.08±0.19 | 0.06±0.13 | 0.05±0.12 |
| 25. | PI 316815 | 10 | 1 | 20 | 1.20±0.45 | 1.14±0.32 | 0.12±0.27 | 0.12±0.27 | 0.09±0.20 |
| 26. | PI 356131 | 10 | 1 | 20 | 1.20±0.45 | 1.12±0.27 | 0.11±0.25 | 0.10±0.22 | 0.08±0.18 |
| 27. | PI 382537 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| 28. | PI 382556 | 10 | 0 | 0 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| Overall | 280 | 3 | 60 | 1.80±0.82 | 1.25±0.34 | 0.24±0.29 | 0.01±0.20 | 0.16±0.20 | |

**Table 6. Group-wise allelic frequency per locus generated by 5 STMS primers**

| S. No. | Accession | HVBKAS1 | | HVM9 | BMS90 | | HVM43 | | | HVM6 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Allele A | Allele B | Allele A | Allele A | Allele B | Allele A | Allele B | Allele C | Allele A |
| 1. | Group I (UA & Nepal) | 0.08 | 0.92 | 1.00 | 0.32 | 0.68 | 0.01 | 0.98 | 0.006 | 1.00 |
| 2. | Group II (J&K) | 0.62 | 0.38 | 1.00 | 0.72 | 0.28 | – | 1.00 | – | 1.00 |
| 3. | Group III (HP) | 0.35 | 0.65 | 1.00 | 0.20 | 0.80 | – | 1.00 | – | 1.00 |
| 4. | Group IV (Ethiopia) | – | 1.00 | 1.00 | – | 1.00 | 0.14 | 0.86 | – | 1.00 |
| | Overall allelic frequency | 0.26 | 0.74 | 1.00 | 0.31 | 0.69 | 0.04 | 0.96 | 0.002 | 1.00 |

UA=Uttarakhand, J&K = Jammu & Kashmir, H.P.= Himachal Pradesh

**Table 7. Group-wise summary diversity analysis of selected barley landraces**

| S. No. | Group | Sample size | No. of polymorphic loci | % of polymorphic loci | Observed no. of alleles | Effective no. of alleles | Shannon's Information Index | Observed heterozygosity | Expected heterozygosity |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Group I (UA and Nepal) | 170 | 3 | 60 | 1.80±0.84 | 1.19±0.33 | 0.20±0.26 | 0.008±0.18 | 0.13±0.18 |
| 3. | Group II (J&K) | 50 | 2 | 40 | 1.40±0.55 | 1.32±0.44 | 0.25±0.35 | 0.007±0.01 | 0.18±0.24 |
| 5. | Group III (HP) | 20 | 2 | 40 | 1.40±0.55 | 1.26±0.38 | 0.22±0.32 | 0.00 | 0.16±0.22 |
| 6. | Group IV (Ethiopia) | 40 | 1 | 20 | 1.20±0.45 | 1.06±0.14 | 0.08±0.18 | 0.06±0.12 | 0.48±0.11 |
| | Overall | 280 | 3 | 60 | 1.45±0.59 | 1.20±0.32 | 0.19±0.28 | 0.02±0.01 | 0.23±0.19 |

UA=Uttarakhand, J&K = Jammu & Kashmir, HP= Himachal Pradesh

clusters. This provides the opportunity to select diverse accessions from distinct microclimatic niches for specific morphological adaptations. Grouping of accessions from all regions in sub-cluster Ib indicates preponderance of widespread common alleles in barley landraces under study. The AFLP markers have also been successfully used for cultivar identification and genetic diversity analysis in barley (Ellis *et al.*, 1997; Schut *et al.*, 1997; Powell *et al.*, 1997; Davilia *et al.*, 1998; Treuren and van Hintum, 2001; Turpeinen *et al.*, 2003).

**Table 8. Summary of group-wise F-statistics and gene flow for all loci in selected barley landraces [See Nei (1987) Molecular Evolutionary Genetics (p. 159-164)]**

| Group | Sample Size | Fis | Fit | Fst | Nm* |
|---|---|---|---|---|---|
| Group I (UA & Nepal) | 170 | 0.803 | 0.934 | 0.5808 | 0.180 |
| Group II (J&K) | 50 | –16.466 | 0.962 | 0.7269 | 0.094 |
| Group III (HP) | 20 | –16.301 | 1.000 | 0.0581 | 4.056 |
| Group IV (Ethiopia) | 40 | –81.939 | –0.159 | 0.1621 | 1.293 |
| Mean | 280 | 0.755 | 0.920 | 0.675 | 0.120 |

* Nm : Gene flow estimated from Fst : 0.25(1 - Fst)/Fst.
UA: Uttarakhand, J&K : Jammu & Kashmir, HP: Himachal Pradesh

### STMS Characterisation

At the level of the population, any particular allele at a locus occurs with a certain frequency and in self-fertilizing species like barley may attain any frequency. The amount of polymorphism within populations, the allelic richness (the total number of alleles in the population), the gene diversity or probability that two random copies of the gene will have dissimilar alleles, and the heterozygosity (the percentage of heterogeneous genotypes in a population) are all measurements of genetic diversity within a population. The informative value of microsatellite markers for genetic studies and as a powerful tool for linkage maps and barley breeding was confirmed in several studies (Saghai-Maroof *et al.*, 1994; Becker and Heun, 1995; Liu *et al.*, 1996; Struss and Plieske, 1998; Ramsay *et al.*, 2000; Pillen *et al.*, 2000; Kunzel *et al.*, 2000; Kunzel and Waugh, 2002; Holten *et al.*, 2002; Li *et al.*, 2003; Thiel *et al.*, 2003).

Among the five STMS loci, two (HVM9 and HVM6) were entirely invariant as only one allele was observed in the total 280 individuals from 28 populations analysed (Table 4). The alleles at these loci can be categorised as common and widespread according to Marshall and Brown (1975). Of the three remaining loci, BMS90 was most variant followed by HVBKASI and HVM43. All the alleles at these loci occurred with frequency $P>0.1$ for individual populations. At locus HVBKASI, allele A was common and localized and was present in only one population (IC 260864) of the 16 populations of Uttarakhand region studied. This allele was, however, common and widespread in populations of Jammu & Kashmir and Himachal Pradesh, and also occurred in one population of Nepal (CIHO 3228). The above population from Uttarakhand (IC 260864) was collected from the adjoining overlapping areas of Uttarakhand and Himachal Pradesh. Similarly at locus HVM43, allele A and C were common and localized. Allele A at this locus occurred in two populations of Uttarakhand (IC 406567 and PI 176040) and two populations from Ethiopia (PI 316815 and PI 358131). Allele C was present in only one population of Uttarakhand (IC 362256). The common and localized alleles occur in only one or few habitats, where they normally reach a high frequency. These alleles may be biologically specialized alleles that enhance adaptation only in certain habitats. These are often the class of alleles of most interest to breeders, because breeders are concerned with improving performance in the specialized habitats of their own ecogeographical regions. Widespread, common alleles have almost certainly been introduced into all habitats, whereas introduction of the localized alleles of special habitats is likely to have been sporadic. Such considerations led Marshall and Brown (1975) to suggest that locally common alleles are, at least conceptually, the key class of alleles in formulating sampling strategies, whether for capturing the maximal amount of useful variation within populations (within practical limits of sample size) or determining the distribution of environmentally influenced genetic variation within species. Alleles whose frequencies vary widely from population to population (common and localised categories) appear to be responsible for most of the allelic variation that exists within and among populations of barley landraces studied. Such variations in allelic frequencies are often correlated with readily observable and measurable environmental factors (rainfall, temperature, slope, exposure, soil type, etc.).

The population genetic parameters revealed high allelic richness in four of the 16 landraces from Uttarakhand (IC 260864, IC 356114, IC 398291 and IC 362256) together with high Shannon diversity indices (Table 5). Of the five populations of Jammu & Kashmir, one accession, PI 429885 had high allelic richness together with high Shannon diversity index. The two accessions from Himachal Pradesh displayed relatively high allelic richness and high Shannon diversity index. The one accession from Nepal included in the study had the highest allelic richness (effective number of alleles $1.40\pm0.55$) and Shannon diversity index ($0.28\pm0.38$) of all the accessions studied. Among the four Ethiopian accessions, two accessions PI 416815 and PI 356131 had high allelic richness together with high Shannon diversity indices. Five accessions from Uttarakhand (IC 356122, KC/PS 37, IC-406570, IC-406571, IC-355879), two accessions from Jammu & Kashmir (PI-429889, PI-429907) and two accessions from Ethiopia (PI-382537,

PI 382556), did not show any polymorphism for the five STMS marker loci studied.

Group-wise summary statistics (Table 7) for various population genetic parameters indicated that accessions from Himachal Pradesh (group III) and Jammu & Kashmir (group II) had relatively more number of effective alleles and high Shannon diversity index followed by accessions from Uttarakhand and one accession of Nepal clubbed in group I and the accessions from Ethiopia in group IV. Observed heterozygosity is more for Ethiopian accessions followed by accessions from Uttarakhand and Jammu & Kashmir.

Group-wise F-statistics (Table 8) revealed greater population differentiation in group II and I. On average 68% of variation was apportioned among populations and 32% within populations. The contribution of geneflow was more for two populations from Himachal Pradesh (group III) followed by landrace populations from Ethiopia, Uttarakhand and Jammu & Kashmir.

Understanding the population genetic structure of farmers' landraces may be helpful for *in situ* (on-farm) management of these landraces. Farmers' decisions regarding the size and relative placement of their fields impact significantly on local crop diversity. Fields may be large or small, close together or widely spaced. Depending on the reproductive biology of the crops in question, this structuring can have a range of effects on the genetic diversity of the crops. It is not known whether common landraces used throughout the village or region tend to be more variable than less common varieties. It could be that the differences are related much to character differences (and possibly to the distribution of diversity) and all landraces have about the same allelic richness. Neither is known whether increasing richness of farmer varieties or number of farmer varieties and increasing genetic diversity (allele richness) are positively correlated. It could be that the genetic diversity contained in a few landraces in some villages is similar to the amount of genetic diversity contained in villages with many varieties. To make rational conservation plans, it is important to test whether villages with a few landraces are conserving as much diversity as villages with many varieties. Locally common landraces are those landraces that appear to be particularly important for conservation and particularly interesting for users.

Farmer management of geneflow impacts genetic diversity over time and space. Landraces may hybridize with other farmers' landraces. Farmer management of crop populations according to flowering dates is another action with relevance for geneflow between varieties. When new genetic combinations arise through introgression, farmers select and maintain new combinations under specific situations.

The UPGMA dendrogram presented in figure 4 provides opportunity for selecting distinct populations from different microclimatic niches for use in crop improvement. Diversity analysis will provide information on the genetic distinctiveness of farmer-named varieties. Measurement of diversity can determine whether rare varieties are selected from common ones and whether locally common varieties have the greatest number of locally common alleles for different breeding systems. Diversity analysis at different spatial scale will enable comparisons at community and regional levels, such as whether the crop populations of one village represent all the genetic diversity in the region.

The above population genetic parameters can help measure the average genetic diversity of a field/village/region, the differences in allele frequencies among different populations, and the differences in level of polymorphism among populations.

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