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RESEARCH ARTICLE

Traditional seed management and genetic diversity in barley varieties in high-hill agro-ecosystems of Nepal

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Abstract Chawali and Lekali are two common farmer's barley varieties or landraces in Jumla, Nepal (2,240–3,000 m) with small to bold grains and wide adaptation from irrigated low lands to high hills. This study was undertaken to test whether features of the traditional seed system can significantly influence the diversity of a crop and its conservation on-farm. In Jumla (high-hill), the barley seed system is completely informal and is mainly from farmer to farmer. In the present study, the seed flows and the pattern of genetic diversity in barley were investigated to detect differences between the two varieties and test the divergence among populations of each variety These data suggested that Chawali, the more common variety, was less subject to homogenising gene flow between farms than was Lekali. A total of 128 farming households were surveyed for seed supply information and 128 populations for each landrace from two villages: Kartikswami and Talium were collected for SSR diversity analysis. Some 92 SSRs were screened in an initial sample of 20 barley populations of both landraces and 2 improved varieties (LG-51 and Soluwa). Of the 81 SSRs that consistently amplified, only 15 SSRs (19%) were polymorphic with gene diversity values ranging from 0.09 to 0.71. A medium to low diversity was detected among the landrace populations of barley varieties. Chawali populations were less polymorphic within ecological groups, and more divergent between than were Lekail populations. This result accords with Chawali having a more conservative local seed system.

Keywords *Hordeum vulgare* · Simple sequence repeat · Genetic diversity · Polymorphic loci · Informal seed system

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Introduction

Barley (*Hordeum vulgare* L.) is a winter self-pollinated cereal grain belonging to grass family Poaceae. It is grown in different cropping patterns in both *Khet* (irrigated) and *Bari* (non-irrigated) conditions of Nepal. Barley grows in the harsh environment of the high-hills of Nepal, where landraces dominate its cultivation. For those farmers, it is the second preferred crop after rice (Baniya et al. 1997). Earlier studies and explorations in the high-hills revealed considerable diversity in barley germplasm (Witcombe 1975; Witcombe and Gilani 1979). Jumla, our study site is located at 29° 12.5′N to 29° 20′N and 82° 05′E to 82° 10′E and at altitudes from 2,240 to 3,000 m with cool temperate to alpine climate. This district has seen little intervention of modern technology, traditional landraces are well adapted and is prone to food deficit in these regions (Bajracharya et al. 2006, 2010). In Jumla. barley occupies 2.5 times more area than rice, and provides better nutrition and food security for the harsh environment. *Chawali, Bhuwali, Lekali* and *Pawai* are four farmers' varieties and they are all six-rowed varieties grown in a range of agroecological environments in this district. These landraces vary in their morphological traits, in traditional farming system, and for preferred traits and uses (Paudel et al. 1998; Rana et al. 2000). *Chawali* and *Lekali*, the dominant of the four, are two six-rowed distinct landraces, selected for the study. These are grown in large areas and by many farmers. Field and household surveys have found that *Chawali* is mainly cultivated in irrigated rice fields in winter, whereas *Lekali* is grown in diverse conditions from lowlands in rice field to bari (upland) on hilltops and terraces under rainfed conditions, *Chawali* was the common landrace grown for its good quality flour. It is early maturity and spikes are short and small in size and easy to thresh. *Lekali* is grown by resource-poor farmers in less favourable areas (Rana et al. 2000). It is late maturing but high yielding with long spikes, bold grains and widely adapted to a range of environments.

Local crop diversity of barley is likely to be in a state of flux, influenced by the population biology of crop itself, environmental and social aspects, farmer's knowledge and the circumstances of the local seed system (Brown 2000). Various techniques have been used to measure genetic diversity. Indigenous barley varieties of Nepal have so far been studied morphologically (Witcombe and Gilani 1979; Baniya et al. 2003; Gupta et al. 2003), and a limited number of Himalayan collections have been assessed for their isozyme polymorphism (Konishi and Matsuura 1991; and Bajracharya et al. 2003). Recently, DNA technology has greatly influenced studies of crop genetic diversity with the development of different types of molecular markers. Of the available techniques, microsatellites or simple sequence repeats (SSRs) are highly informative, reproducible, codominant, abundant, and demonstrate a high degree of allelic variation and are being extensively employed in barley genetics (Kleinhofs et al. 1993; Pillen et al. 2000). No diversity analysis that relates to systems of traditional barley seed management has been performed at the molecular level. The objective of the present study is therefore to determine the effect of the local seed system in Jumla (high-hill) Nepal on the genetic structure of common barley landraces using surveys, seed collection and simple sequence repeat (SSR) markers. In particular, populations of the more widely adapted, but rarer *Lekali* might be expected to diverge more than those of the commoner *Chawali*, but high levels of seed exchange could outweigh such divergence.

Materials and methods

Sites for survey and sample collection

Talium (2,600 m) and Kartikswami (2,620 m) were two villages in Jumla chosen for survey and seed sampling during the harvest period of 2005. Based on the altitude range, temperature, nature of irrigation: *Gadkule* (snow-melted river) and *Kholapane* (stream water) and orientation of land, five agroecological environments each for *Chawali* (1–5) and *Lekali* (6–10) (Table 1; Fig. 1). In general, *Khet* (irrigated land) and *Bari* (rain-fed upland) lands in the study area are medium to poor in soil fertility and *Lekhs* (high-hill agricultural fields far away from the owner's homestead) are with good status. *Chawali* were collected at lower elevations whereas *Lekali* samples came from the upper regions of the selected sites (mostly from *Lekhs*). From the total households (HHs) within each of these environments we selected randomly 8–18 farming HHs. For the seed system

Table 1 Agro-ecological groupings of the site for collection of *Chawali* and *Lekali* barley seed samples

Group number	Average village altitude	Aspect	Others	Village name
1	Low	North	River water	Khalla Silam
2	Low	South-East	River water	Sridhuska, Bayalkatiya
3	Low	East	Stream water	Siyalbada
4	Low-medium	West	Stream water	Talium, Rokayabada, Dharalabada
5	High	South	Stream water	Jantebhir
6	Low	East	Rain-fed	Bayalchour, Tarekhola
7	High	East	Rain-fed	Sarapani-Maldada
8	High	South-East	Rain-fed	Dungri-Dahanta, Simsada-Mahadev
9	High	East	Rain-fed	Ranfe
10	High	West	Rain-fed	Lada, Kotha

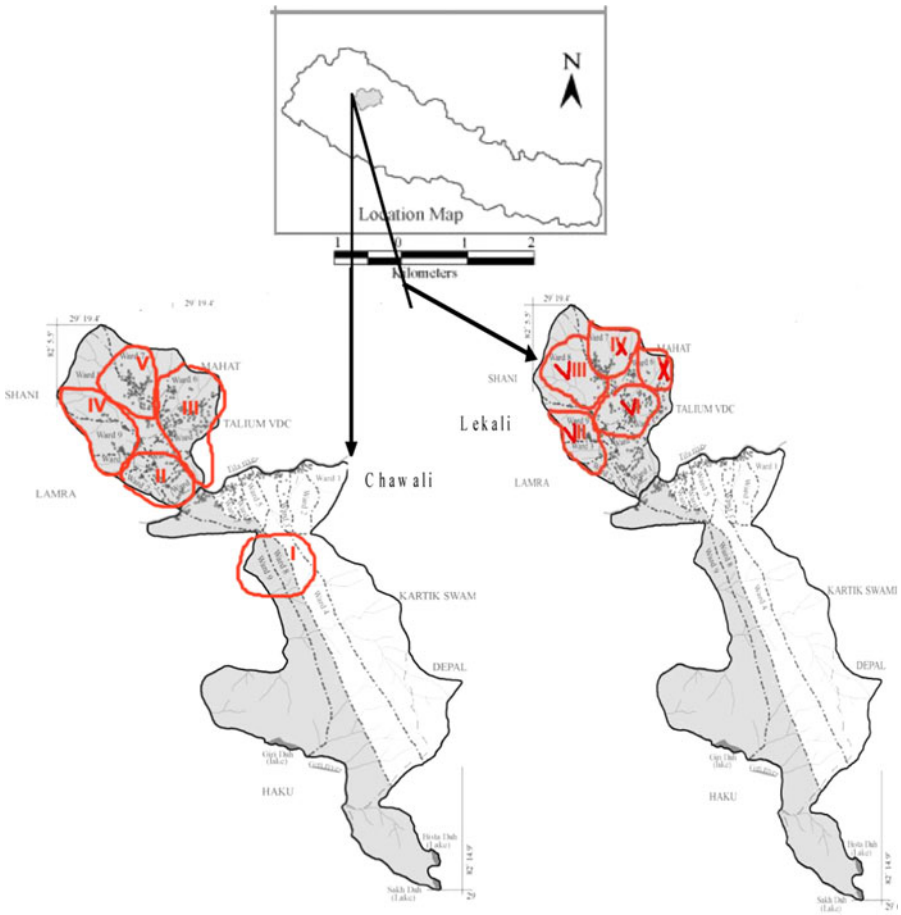

Fig. 1 Site map of the *Talium* and the Northern parts *of Kartik Swami* VDC in Jumla, Nepal identified for seed survey of barley landraces

data on each variety, 64 farming households (HHs) were surveyed. Some 34 HHs were growing and maintaining both the varieties, so that the total number of HHs surveyed was only 94. Seed samples were collected from two fields of each farmer for each variety, and treated as separate populations because often the fields of that farmer were spatial separated. A total of 256 seed samples were collected in the survey and each sample represented a population.

Seed management

The farms were surveyed for a range of indicators of seed management system. The data related to indicators were collected from farmers through interviews following a standard questionnaire. The information was therefore for each household about each variety under cultivation and included the following:, the source of seed lots, the frequency of seed exchange among farmers, the frequency of replacement (including all sources of new seed), whether the current planting was of fewer varieties, the same number, or more varieties than that farmer had grown recently, and the farmer's belief about seed availability in the local market. The data were acquired during the barley planting season of 2005 to understand the pattern of seed migration.

Plant materials for SSR diversity

From the total collected samples, 40 barley populations for each of the two varieties were included in the molecular study. For this purpose, four farms from each of the five collections environments (groups) were chosen and for each of these, two populations were chosen for the study. The improved vareties LG-51 and Soluwa were used as controls. Seeds of these control varieties were provided by the Hill Crop Research Programme (HCRP), NARC, Kavre, Nepal. The sampling design therefore stratified the materials according to landrace, collection environments, farmers, and farmer's fields, giving a total of 80 landrace populations for study (Table 2).

DNA extraction

Two spikes were randomly selected from each population for DNA source. Five seeds from each selected spikes were raised in plastic pots in a growth chamber for about 3–4 weeks. One seedling from each of two random spikes of a population was used for individual DNA extraction. From 100 to 200 mg of fresh green leaf tissue of individual seedlings was used for genomic DNA extraction following the modified CTAB method (Roger and Bendich 1988). The quality and quantity of isolated DNA was determined on 0.8% agarose mini-gels in 1× TBE buffer (0.09 M Tris–borate and 0.5 M EDTA) at 80 V for 90 min with ethidium bromide staining by comparing bands to known concentrations of lambda DNA.

SSR analysis

A total of 92 genomic microsatellite markers (gSSRs) and expressed sequence tag-derived microsatellite markers (EST-SSRs), including all barley chromosomes were assayed on the populations (MWG-Biotech AG, Paisley,UK). The websites: http://www.scri.sari.ac.uk/ssr; and http://www.wheat.pw.usda.gov/ggpages/SSR provided a list of well characterised barley SSRs as genetic markers developed by Scottish Crop Research Institute (SCRI), Dundee. These primers are available worldwide as a common set of robust PCR-based tools (Saghai Maroof et al. 1994; MacCauley et al. 2001; and Matus and Hayes 2002). From the list of SCRI and other previous reports by Becker and Heun (1995), Roder et al. (1995), Lui et al. (1996), Russell et al. (1997), and Ramsay et al. (2000). They are of 17–26 mer genomic and expressed sequence tags (EST)SSRs of barley and one SSR marker of wheat with high polymorphism. These markers were screened for polymorphism and optimization of protocol with local barley landraces of Nepal.

PCR reactions were performed in a total volume of 15 µl containing 20–100 ng of DNA, 0.25 µM of

Table 2 Number of farming households, number of barley populations and their ecological clusters (growing environments) selected for SSR diversity analysis

No of farmers	*Chawali*		*Lekali*		Total
	Populations (no)	Groups	Populations (no)	Groups	Populations
4	8	1	8	6	16
4	8	2	8	7	16
4	8	3	8	8	16
4	8	4	8	9	16
4	8	5	8	10	16
20	40	5	40	5	80

each primer, and Reddy Mix™ PCR Master Mix 3.0 mM $MgCl_2$ (ABgene, Epsom, Surrey, UK) and processed for amplification in a MJ Research PTC-100™ programmable Thermal Controller with Hot Bonnet (MJ Research, Inc., Waltham, MA, USA) following a touchdown PCR programme. The touchdown thermal cycling comprised of 38 cycles of 94°C for 1 min denaturing and 72°C for 1 min extension. Annealing temperatures each for 30 s were progressively decreased by 1°C every second cycle from 64 to 55°C at which temperature they remained constant for final 30 cycles. The amplification ended with a final 5 min extension at 72°C and infinite hold at 4°C. PCR amplification products were detected on a 3% horizontal agarose gel electrophoresis using Midi ABgarose (ABgene, Epsom, Surrey, UK) stained with ethidium bromide for 4 h at 90 V. The products were visualized under UV illumination, photographed with a 'Gel Cam' and sized with GeneScan.

Data analysis

In virtually all cases, a single prominent product was resolved for each primer pair. The SSR and EST-SSR data were scored as alleles defined by a particular fragment size class for the SSR marker locus, The values for gene diversity were calculated for each genomic SSRs and EST-SSR markers [for the jth locus GD(j)] according to the formula: $GD(j) = 1 - \Sigma_i(P_{ij})^2$, where P_{ij} is the frequency of the ith different fragment size revealed by the jth primer summed across all patterns revealed by the primers (Botstein et al. 1980). The overall GD was computed as the average of all the GD(j) values. For measurement of genetic similarity among samples, each allele was converted to a presence versus absence character, and the entries in the genetic similarity matrix calculated as proportion of shared fragments (Nei and Li 1979). Principal component analysis was performed using the NTSYS-pc version 1.80 software (Rohlf 1993).

Results

Seed management of two landraces in Jumla

A preliminary report on the complete interview data on seed supply system of barley in Jumla was presented elsewhere (Bajracharya 2005; Joshi et al. 2009). Here we summarise the data for the 25 farmers included in the molecular diversity study, 15 of whom grew both the landraces and 5 farmers each grew either *Chawali* or *Lekali*. Most the farmers' seed lots (populations) of both the landraces were from their own saved seed. However, 25% farmers for *Lekali* and 20% for *Chawali* exchanged seed among the farmers of their village. They obtained seed in the form of a loan, or bought from other farmers. For variety *Lekali*, 65% of farmers adopted seed renewal practices while renewal was only 25% for *Chawali* (Table 3). The rest of the farmers kept seed exclusively from their own previous harvest.

Screening of SSR primers in representative samples of barley

Among 92 genomic and EST derived microsatellite (SSR) markers screened, 81 reliably amplified the barley DNAs, and only 15 loci (19%) were found polymorphic with diversity values ranging from 0.09

Table 3 Analysis of seed variables surveyed in study sites of Jumla

Variable	State	Alternative	*Chawali*	*Lekali*	Chisquare probability
Knowledge of selling and buyer	Yes	No	0.53	0.47	0.43
Variety richness changes	Increasing	Decreasing or static	0.11	0.20	<0.05
Area of cultivation	Decreasing	Increasing or static	0.17	0.05	<0.05
Participated in seed exchange within last 3 years	Yes	No	0.24	0.28	0.52
Seed replacement or renewal within last 3 years	Yes	No	0.42	0.93	<0.001
Kind of exchange	As loan	Purchased	0.56	0.08	<0.001

Table 4 SSR markers used in diversity analysis with the non-zero GD values in 20 representative samples of barley landraces from Jumla and 2 check varieties: LG1 and Soluwa

S. no	Loci	Chromosome	Published GD	Repeat motif	Number of alleles	Observed GD
1	Scssr07759	(2H)	–	–	4	0.71
2	HVM5	1 (7H)	–	(GT)6(AT)16		0.41
3	HVM14	6 (6H)	–	(CA)11		0.39
4	HVM49	1 (7H)	–	(CA)12		0.29
5	HVCSG	2 (2H)	–	(CA)4(C)17		0.28
6	HVDHN7	7 (5H)	–	(AAC)5		0.26
7	Scssr09398	(6H)	–	–		0.24
8	Scssr14079	4 (4H)	0.4	–		0.16
9	HVM54	2 (2H)	0.78	(GA)14	2	0.09
10	Bmac0067	3 (3H)	0.82	(AC)18	2	0.09
11	Bmag0009	6 (6H)	0.59	(AG)13	2	0.09
12	Scssr15334	7 (5H)	nd	–	2	0.09
13	HVM27	3 (3H)	0.66	(GA)14	2	0.09
14	HVM62	3 (3H)	0.78	(GA)11	2	0.09
15	HVM65	6 (6H)	0.68	(GA)10	2	0.09

(the lowest nonzero value) to 0.71 (for Scssr07759) in the 22 samples. The polymorphic loci were nine genomic SSRs (all labelled HVM in Table 4) and six EST-SSRs (labelled Bmac, Bmag or Scssr in Table 4). These loci represent all chromosomes except the one, and include di- and tri-nucleotide repeat motifs. Most of these loci had two alleles, the highest number of alleles was four for Scssr07759. Overall the 81 SSRs tested, including the 66 monomorphic loci, the average GD and allelic richness values were 0.042 and 1.3 respectively.

Pattern of genetic values for gene diversity in Chawali and Lekali seed lots

Five SSR loci: HVM5, HVM14, HVDHN7, Scssr 07759 and Scssr 09398 were assayed on all 160 individual DNAs of 80 (40 each of *Chawali* and *Lekali*) individual farmers' seed lots and the two check varieties: LG1 and Soluwa. HVM54 and HMV65 failed to amplify products for many of the barley populations studied, so they were excluded. A total of 15 alleles was detected across five SSR loci

Fig. 2 Microsatellite DNA fragments detected with Scssr 07759 showing alleles in 80 individual DNAs of *Chawali* populations of 20 farmers collected from 5 ecological clusters in Jumla. (In each plate the *lanes* 17, 18 and 19 from *left to right* represent the ladder, LG1 and Soluwa respectively)

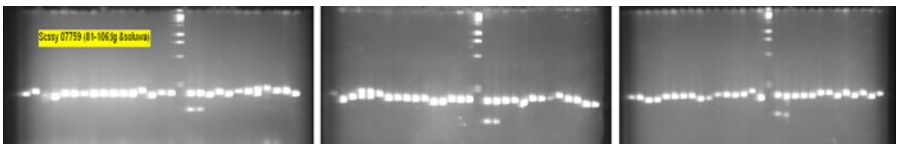

Fig. 3 Microsatellite DNA fragments detected with Scssr07759 showing alleles in 80 individual DNAs of *Lekali* populations of 20 farmers collected from 5 ecological clusters in Jumla. (In each plate the *lanes* 17, 18 and 19 from *left to right* represent the ladder, LG1 and Soluwa respectively)

Table 5 Genetic diversity (GD) values for different populations of barley landraces

Polymorphic SSR markers	*Chawali* groups					*Lekali* groups					Genetic diversity
	1	2	3	4	5	6	7	8	9	10	
Scssr07759	0.76	0.60	0.53	0.49	0.12	0.50	0.57	0.55	0.54	0.40	0.70
Scssr09398	0.00	0.00	0.00	0.22	0.00	0.14	0.26	0.12	0.34	0.20	0.23
HVM 14	0.00	0.00	0.00	0.00	0.22	0.50	0.47	0.44	0.49	0.43	0.37
HVM 5	0.46	0.00	0.49	0.00	0.00	0.00	0.48	0.00	0.00	0.00	0.50
HVD HN7	0.36	0.00	0.00	0.44	0.12	0.36	0.50	0.32	0.31	0.47	0.52
Average GD	0.39	0.15	0.26	0.23	0.09	0.30	0.46	0.36	0.42	0.37	0.46

with an average three alleles per locus in the whole set of 80 barley populations. Of the polymorphic loci assayed, HVM5 and HVM14 had two alleles per locus, HVDHN7 and Scssr09398 had three alleles and Scssr07759 detected the highest number of five alleles per locus over the whole populations of *Chawali* and *Lekali* under study (Figs. 2, 3). In all assays only a single amplification product was obtained, implying complete homozygosity for all loci, and no evidence of recent outcrossing. The total number of alleles detected at these loci in *Chawali* was 15, and in *Lekali* 13. A moderate diversity among farmers' seed populations of two varieties from five different environmental clusters in Talium and Kartikswami was detected for these SSR loci. Two alleles at the loci (Scssr07759 and HVDHN7) were unique to *Chawali* variety, and in fact unique to a single farmer's population (<0.05) respectively.

The GD values for the five loci were compared between the two varieties and among the populations and the ecological clusters of sites (Table 5). Considering the entire barley populations (80) of *Chawali* and *Lekali*, the GD value ranged from 0.23 (Scssr09398) to 0.70 (Scssr07759) with the average value of 0.46. The average GD for the *Chawali* populations was 0.30, while the average for *Lekali* populations was 0.38. Within *Chawali,* GD ranged from 0.05 (HVM 14) to 0.64 (Scssr07759) and for *Lekali*, it was from 0.10 (Scssr09398) to 0.62 (Scssr07759) (Table 5). Scssr07759 a contig indel type was most informative for the populations in the study.

A moderate level of diversity was found within the barley populations belonging to each of environmental collection groups for both the varieties. Based on (GD) values, for *Chawali* the group 5 was least polymorphic (GD = 0.09) and group 1, the most diverse (GD = 0.39) (Table 4). Likewise, for *Lekali*, the groups 7 and 9 were found to be composed of genetically variable populations (0.46 and 0.42) respectively. The five-locus average GD values for *Chawali* groups tend to be lower than those for the *Lekali* groups of samples (Mann–Whitney *U* Test probability, $P < 0.05$). A rank test of all 50 single GD values in Table 5 confirmed the statistical significance ($P < 0.01$) that GD values within ecological groupings were lower for *Chawali* than for *Lekali.*

A two-dimensional plot of the PCA classifying the 80 barley populations based on allelic variation is shown in Fig. 4. The first principal component axis accounted 32% of the total variation and the second accounted 12% of the total variation. In the PCA the *Lekali* populations were largely separate from the *Chawali* populations by these two axes. With few exceptions, *Lekali* tended to be negative for the *X*-axis and positive on the *Y*-axis, whereas *Chawali* were either positive on the *X*-axis or negative on the *Y*-axis. The contingency chi-square value of this divergence between the two varieties (2 varieties × 4 quadrants) was highly significant (>49; $P < 0.001$). The PCA exhibited genetic variation among the populations within and between the varieties in the study. The *Lekali* populations were less genetically differentiated, and were clustered more closely together while the *Chawali* populations were dispersed, particularly for the *X*-axis (Fig. 4).

Discussion

Determining the amount of genetic variation and the relationships among farmers' populations of landraces requires that the markers exhibit some degree of polymorphism (Yee et al. 1999). This study

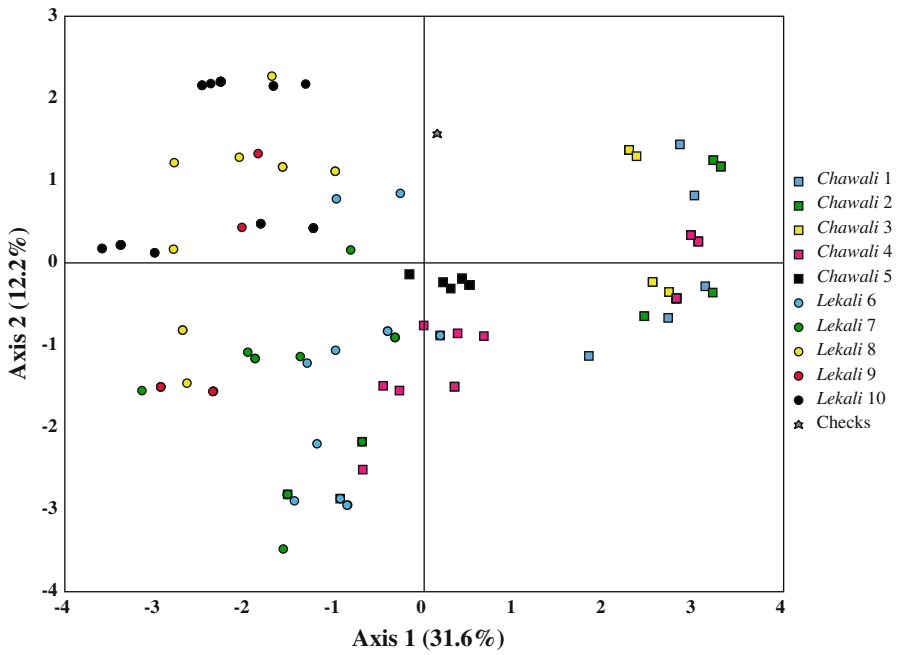

Fig. 4 *Scatter plot* of first two components for 40 individual populations each of *Chawali* and *Lekali* landraces and two check varieties (LG1 and Soluwa) showing 44% of total alleles variation measured at 5 polymorphic loci

employed allelic variation at SSR loci to detect genetic divergence among and within farmers' populations of two barley landraces (*Chawali* and *Lekali*). Eighty populations from each variety were sampled from an altitudinous area (Jumla, Nepal) covering 10 distinct production environments. Of 92 gSSRs and EST-SSRs screened, these populations exhibited polymorphism only at 15 loci, of which only five loci (HVM5, HVM14, HVDHN7, Scssr07759 and Scssr09398) were useful for the full sample.

Landraces are populations that farmers manage over time through a sequence of cropping seasons, and become adapted to their local environments. The seed survey in Jumla for barley found that most farmers normally keep seed stocks of both the landraces, replace seed only following a crop failure, and rarely practice seed selection. For the source of replacement seed, they mainly exchange the seed with their neighbours, but a few obtain it in the form of loan. Depending on the resources of the individual farmer, barley landraces are rarely grown in many plots. They are grown in separate fields either in adjacent fields, or in fields far away from the homestead. Most of the farmers of the 80 barley populations included in the genetic study maintain both landraces. *Lekali* is more frequently replaced than *Chawali* and more often with market than neighbour's seed. Overall, the data statistics indicate that the seed system for *Lekali* with greater replacement might feature more homogenising gene flow among farms, than that of *Chawali*. Thus, as a metapopulation system, *Lekali* populations would appear to undergo local extinction and the recolonization from presumably fewer and therefore more uniform sources. If this is the case, then local populations of the *Chawali* system would be more isolated from one another, and, other things being equal, more liable to diverge from one another. This was indeed the case (Fig. 4). Here is comparative evidence of the impact of seed system on the population genetic structure of barley landraces.

The study estimated the degree of similarity among the barley populations. The data indicate that these landrace populations are variable between and within the production environments, as would be expected if these populations are dynamic gene pools that evolve over time in response to production environment and natural selection. Our analysis showed that there was a greater similarity between the *Chawali* populations than between the *Lekali* populations. It could be reasoned that the *Chawali* is common and is continuously grown in large areas and maintained by most farmers whereas resource-poor farmers maintain *Lekali*, growing it in small fields. *Lekali* is widely adapted even in harsh environment.

The result supports the findings of Busso et al. (2000) in pearl millet that the level of variation among individual farmer populations could vary significantly and number of varieties is not the only factor for diversity. The frequency of seed exchange and with whom and where the exchange took place are likely factors that affect the degree of divergence between the several populations of a landrace. A similar observation was encountered for *Lekali*, too. Cluster 9 was more diverse than rest and most farmers in this cluster were maintaining only the populations.

Genetic distances varied considerably between different pairs of populations, and ranged from 0.0 to 1.0, with an overall mean of 0.55. This variable divergence could arise from continuous adjustment to changing environments, variable traditional practices of seed replacement and exchanges of seed among the farmers and villages ($P < 0.001$); or due to small experimental sample sizes. The results of this study thus suggest the existence of a considerable level of diversity in terms of allelic variability among farmers' populations of differently named barley landraces. No clear grouping was evident of the populations by geographical or production environments in either of the landraces under study. Overall, the study found a low percent of polymorphic loci as might be expected for barley populations maintained in marginal habitats in high hill ecosystem.

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