



# On-farm conservation of agricultural biodiversity in Nepal

Volume I. Assessing the amount and distribution of genetic diversity on-farm

*Proceedings of the Second National Workshop 25–27 August 2004, Nagarkot, Nepal*

**B.R. Sthapit, M.P. Upadhyay, P.K. Shrestha and D.I. Jarvis, editors**



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Introduction: assessing the amount and distribution of genetic diversity

## **The amount and distribution of local crop diversity in three eco-physiographic regions of the *In Situ* Conservation Project in Nepal**

*Sanjaya Gyawali, Bal K. Joshi, Parsu R. Biswokarma, Anthony H.D. Brown, D. Jarvis and Bhuwon R. Sthapit*

### **Abstract**

The basic diversity data of landraces has been found important to monitor the dynamics of crop genetic resource management. The diversity data generated in time and space have been valuable to communities, scientists and policy managers to formulate and implement conservation strategies of *in situ*, on-farm as well as *ex situ* conservation and management of genetic resources. In this paper, we document and summarize the basic diversity data of eight mandate crops of *in situ* conservation of the agrobiodiversity project in Nepal. We used various parameters of diversity at varietal and genetic levels that had been generated during the project period (1998–2005). The varietal richness, evenness or dominance of landraces was estimated using diversity indices. Similarly, diversity measured at gene level using isozymes and SSR markers is also summarized here. This documentation has been seen as important information for scientists to monitor the crop genetic resources on-farm and for policy managers to develop long-term conservation strategies.

**Key words:** Agrobiodiversity, diversity data, *in situ* and on-farm conservation, Simpson index, molecular marker, Nepal

### **Introduction**

Crop genetic resources can be conserved by two approaches: *in situ* (on-farm, in its place of origin) or *ex situ* (off-site, outside its place of origin) as in botanical gardens, field genebanks and seed genebanks. On-farm (*in situ*) conservation of landraces refers to plants or their wild relatives that are conserved in the very place where they developed their present-day characteristics. In on-farm conservation, the entire agroecosystem is conserved, including cultivated crops as well as their wild and weedy relatives that may be growing in nearby areas (Brush 1995). The simplest solution to the storage problem is to maintain wild and cultivated traditional landraces in their natural habitats as genetic storehouses. Effective on-farm conservation requires knowledge of the variability patterns of the crop species and their ranges of adaptation (Brown 1978). The variability patterns of landrace diversity indices consist of two parts: richness, which represents the number of landraces in a community, and evenness, representing the relative abundance of the individuals among the various landraces present in the community (Frankel *et al.* 1995).

Landrace ‘richness’ is determined by counting the number of landraces in the community, which is a simple and effective tool to estimate the diversity (Powers and McSorley 2000). The richness is one part of the diversity measurement whereas the evenness or dominance of landraces expresses distribution of landraces in community. We used ‘dominance’ of landraces, which is the opposite of the diversity concept, and refers to the degree that a community is dominated by particular or a few landraces. The Simpson index measures the dominance of the landraces whereas the reciprocal of the Simpson index is the measure of the diversity. The molecular techniques using isozymes and molecular markers (in our case SSR markers) are mostly employed to measure the diversity at genetic level and an attempt has been made to summarize the information generated through molecular markers. These tools have been found extremely useful for

looking at diversity at the lowest level and are therefore helpful in developing conservation and management strategies.

In the *in situ* conservation of agrobiodiversity project in Nepal, the project teams have completed various studies to measure diversity at varietal and gene levels. This paper documents and summarizes the amount and distribution of diversity data of mandate crops from various studies conducted during the first and second phases of the project: 1998–2004. The mandated crops include four self-pollinated crops (rice, barley, finger millet, pigeonpea), two open-pollinated (sponge gourd, cucumber), one clonal crop (taro) and one with both cross- and self-pollinated species (buckwheat). The paper aims to present the amount and distribution of selected crop species in three ecosystems of Nepal.

### **Materials and methods**

The diversity data on eight mandate crops are based on baseline information of the global project on “Strengthening the scientific basis of *in situ* conservation of agrobiodiversity on-farm–Nepal” components from late 1997 to early 2005. The baseline information was, in many cases, revalidated with farming communities during the last eight years of the project period before diversity data were summarized. The eight mandate crop species were rice, barley, finger millet, buckwheat, taro, cucumber, sponge gourd and pigeon pea. Three project sites located in three ecosystems, i.e. Talium-Jumla (high mountain valley), Begnas-Kaski (mid-hill) and Kachorwa-Bara (lowland) were studied (Figure 1abc). Rana *et al.* (2000a, 2000b, 2000c) have explained the details of project sites and mandate crops.

We used data of 22 hamlets in Bara and Kaski each and 18 hamlets in Jumla to estimate the various parameters of diversity. Varietal richness and average varieties per household were estimated based on the farmers’ named landraces. On-farm studies have shown that farmers use variety names as a basic unit of diversity for day-to-day on-farm management and often this is consistent at the household level and within the community and villages as they use this information to exchange genetic materials and communicate associated knowledge about the materials (Sthapit and Jarvis 2005). Farmers use a set of descriptors to distinguish the varieties and have specific local names to describe unique morphology, for example for taro in Nepal (Rijal *et al.* 2003). The consistency index of variety names as a proxy indicator of diversity decreased as the distance from referenced villages increased (Sadiki *et al.* 2005). The baseline information, which had been validated several times, was used to calculate Simpson index and to measure the dominance of landraces at household and village (community) levels. We used Simpson index because it is based on proportion of data points which is least affected by zero (an absence of any of the data points). Similarly we also estimated the divergence between farms at community level. The diversity at allelic level was estimated using molecular markers (isozymes and SSR markers) during the project period and is reported in various papers (Bajracharya 2003; Rana 2004).

### **Results and discussion**

#### **Rice (*Oryza sativa* L.)**

This is one of the most studied crops in the project. We found that a total of 618, 303 and 81 ha of land was cultivated by the respondent farmers of Bara, Kaski and Jumla, respectively, indicating that the *terai* (Bara site) ecology has larger rice land followed by middle hills and high hills (Table 1). In Bara, farmers reported 53 rice varieties including 42 local landraces but when we analyzed the area devoted to these landraces it was only 16% of total rice area. The reverse was true in Kaski where we recorded 69 rice varieties including 64 landraces and the area devoted to all these landraces together was also very high (64%). In Jumla, we did not record any modern varieties; 100% of rice area was under local landraces and farmers reported 21 landraces in Jumla. The average landraces per household (HH) was highest in Kaski (2.94) followed by Jumla (1.09) and Bara (0.68).



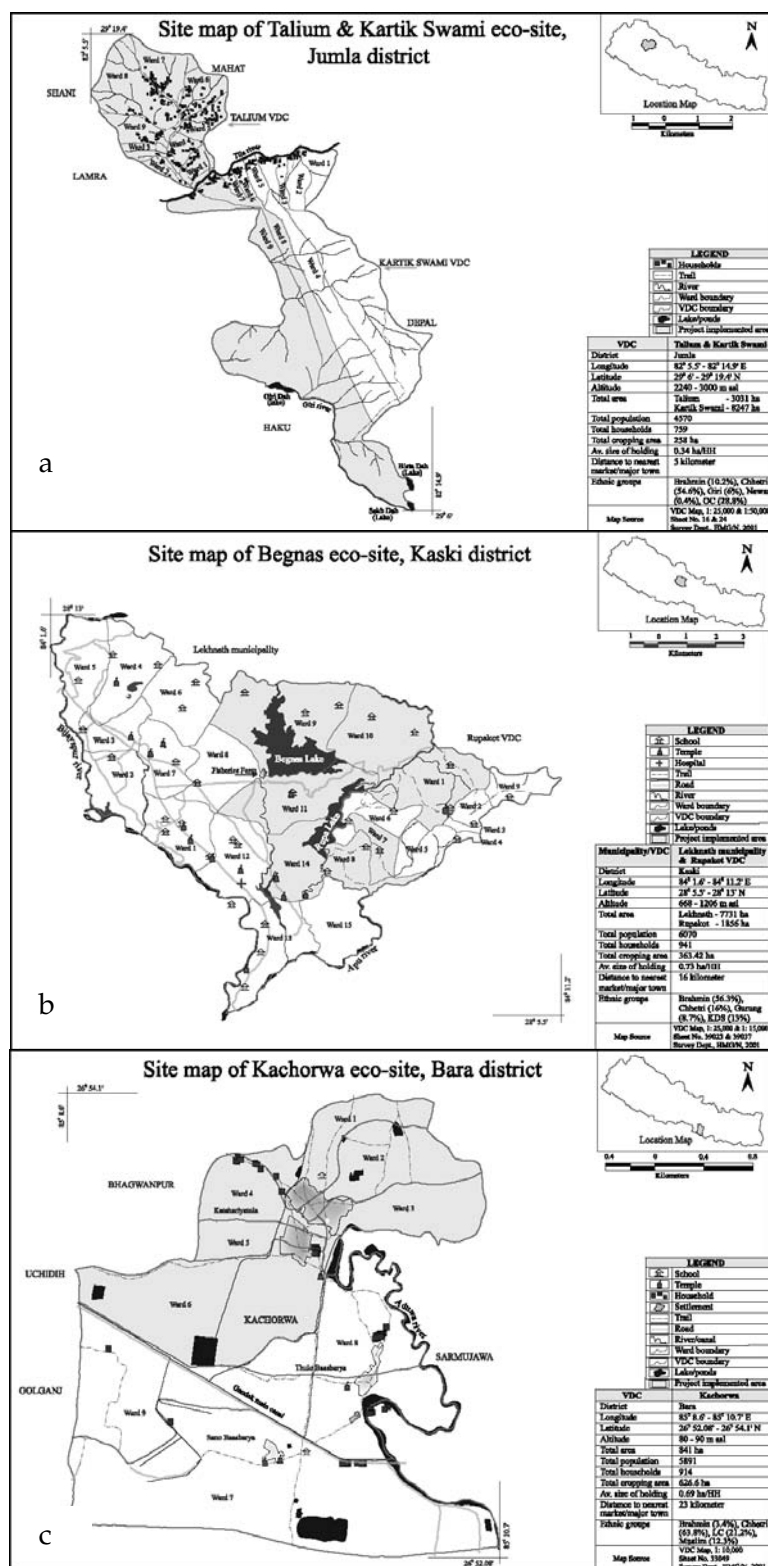


Figure 1. Site maps of three project sites studied. Source: NARC/LI-BIRD/IPGRI 2001.

We analyzed the dominance of landraces using Simpson index at household and community levels based on area under cultivation of landraces at household level. We noticed that a few landraces—particularly *Jethobudho* and *Ekle*—dominated the community in Kaski resulting in a high Simpson index (0.29) compared with a low index in Bara (0.07) and Jumla (0.05) (Table 1). This agreed with the divergence data estimated for each site, indicating higher divergence between farms in Bara (92.5%) and Jumla (93%) than in Kaski (68.4%). These results revealed that although the area under landraces was relatively lower in Bara, the distribution of landraces between farms was more uniform, indicating higher evenness of landraces in Bara and Jumla. The molecular studies conducted using isozymes and SSR markers indicated that Bara and Kaski had the highest average gene diversity (PIC) compared with Jumla (Table 1). Bajracharya (2003) reported the highest polymorphic loci in Jumla followed by Kaski and Bara but the average gene diversity was highest in Bara among all sites.

**Table 1. Comparative amount and distribution of rice landrace diversity on-farm at the *in situ* project in Bara, Kaski and Jumla districts in Nepal, 1998.**

	<b>Jumla</b>	<b>Kaski</b>	<b>Bara</b>
Community Varieties	Talium and Kartikswami 18 (Khalla Silam, Gharti-Kami-Damai, Shreedhuska Bahun, Shreedhusak Thar, Bayal Katiya, Sarki Bada Ka, Sarki Bada Kha, Tallo Rokayabada, Dharala Bada, Talium Thar Bada, Lawar Bada, Rokaya Bada, Damai Bada, Bhandari-Lawar, Budha Bohara, Thapa Lawar, Bhandri Thapa, Siyal Bada)	Begnas 22 (Unnatsil, Devasthan, Mahila Sabisa, Upallo Talbeshi, Rupakot Jamalkuna, Rupa Sundaridada, Kholabeshi, Pourakhe, Chaur, Kalimati, Archalthar, Viveksil Amasamu, Rupa Srijana Ama, Punithar, Poudelthar, Aduwabari, Simalpata, Kotbari, Adhikarithar, Majthar, Dandathar, Bisaunathar)	Kachorwa 22 (Pachiyari Math tol, Mahabir tol, Sarkari Inar tol, Baba Tol, Bording tol, Bajar tol, Math tol, Baluwa tol, Nadi tol, Dhanbhakhari tol, Sadak tol, Chauki tol, Masjid tol, Gola tol, Bhakta Inar tol, Budhuwa Inar tol, Ramana tol, Kamiti tol, Bajarangi tol, Parasnath tol, Purohit tol, Office tol)
Total area (ha) for this crop (total cropped area in parentheses)	81 (258)	303 (363)	618 (627)
No. of modern varieties	0	5	11
Proportion (%) of farm devoted to growing traditional varieties	100	64	16
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	21	69	53
No. of farms in the community/ecosite (total HHs in parentheses)	180 (759)	206 (941)	202 (914)
Avg. no. of variety units per farm (or HH)	1.09	3.61	2.65
Avg. no. of landrace units per farm (or HH)	1.09	2.94	0.68
Avg. evenness or Dominance (Simpson)	0.05	0.29	0.42
Total units or varieties (richness) in the community	21	69	53
Structure – between/within farms	93.0%	68.4%	92.5%
% divergence between sites			
Comments, Caveats	This information was estimated from baseline survey conducted in 1998. Community is groups of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village/farmer group/hamlet–household–farm. We have reported here based on ecosite (see Figure 1abc). Most of the information is derived from published documents.		
Standard Error, CV	SE, 0.1296, CV, 6.103%	SE, 0.481, CV, 7.71%	SE, 1.685, CV, 7.25%

Deeper analysis, basis for the specific analysis: phenotypes, isozymes, microsats, pedigree analysis	Phenotypes	Phenotypes	Phenotypes
Purpose of the study	Microsatellite	Microsatellite	Microsatellite
Number of types (alleles or genotypes) per 'locus' – any measure of richness	Varietal genetic distinctiveness Diversity assessment Phenotypes: 14 quantitative and 14 qualitative traits, Avg. $H' = 0.23$ Microsatellite: 39 SSR markers Total polymorphic loci: 27 Avg. number of alleles per locus: 2.9	Varietal genetic distinctiveness Diversity assessment Phenotypes: 25 quantitative and 14 qualitative traits, Avg. $H' = 0.82$ Microsatellite: 38 SSR markers Total polymorphic loci: 21 Avg. number of alleles per locus: 1.73	Varietal genetic distinctiveness Diversity assessment Phenotypes: 18 quantitative and 14 qualitative traits, Avg. $H' = 0.84$ Microsatellite: 38 SSR markers Total polymorphic loci: 8 Avg. number of alleles per locus: 1.45
Evenness (Simpson or Nei) – can average over morphological characters or alleles	Average gene diversity (PIC) = 0.17	Average gene diversity (PIC) = 0.39	Average gene diversity (PIC) = 0.4
Comments, Caveats	Not all the landraces were studied. We report here only the summary of agromorpho-logical and microsatellite analyses. Sample sizes were not consistent. Data are presented by landraces, not sites. Some data are from on-farm, some from on-station.		

### Barley (*Hordeum vulgare* L.)

Barley is the crop of high-altitude areas and therefore we studied various parameters of diversity of barley in Jumla ecosite. Few farmers grow barley in Kaski ecosite and none in Bara. The diversity data of barley is representative of 18 hamlets of the project site comprising 180 households with 120 ha of barley area (Table 2). We recorded 5 barley landraces with 100% area covered by these landraces. The average landraces/HH was recorded as 1.55 and with a Simpson index of 0.32. We found the divergence between farms was as low as 20.16%. These diversity parameters indicated that there was relatively higher dominance of particular landraces at community level and landraces grown on-farm were therefore less evenly distributed in the community. The molecular analysis using 5 isozymes revealed an average coefficient of 0.149 and average number of alleles per locus of 1.38.

### Finger millet (*Eleusine corocana* L.)

Finger millet is the second most important hill crop after maize in Nepal and is grown under marginal conditions (poor soil fertility, rain-fed/drought and mixed cropping). We studied the diversity of this crop in all three project sites. The finger millet data were collected during baseline years 1998 to 2003. We found that Kaski has the highest finger millet area (133 ha) in the project site followed by Jumla (35 ha) and Bara (0.7 ha) (Table 3). In all sites farmers cultivated only landraces although the Hill Crop Research Program (HCRP) has released a number of varieties in the past few years. Bara farmers grow 6 landraces whereas farmers of Kaski and Jumla maintain a total of 24 and 12 landraces respectively but the average landraces/HH was higher in Jumla site (1.31 landraces/household) followed by Kaski (1.22) and Bara (0.09). The Simpson index of finger millet in Kaski and Jumla was estimated as relatively higher compared with Bara but we noticed that the divergence between farming households was higher in all three cases. These diversity parameters revealed that the landraces were distributed relatively evenly at the community level.

**Table 2. Comparative amount and distribution of barley diversity on-farm at the *in situ* project in Jumla district in Nepal, 1998–2000.**

	Jumla
Community Varieties	Talium and Kartikswami 18 (Khalla Silam, Gharti-Kami-Damai, Shreedhuska Bahun, Shreedhusak Thar, Bayal Katiya, Sarki Bada Ka, Sarki Bada Kha, Tallo Rokayabada, Dharala Bada, Talium Thar Bada, Lawar Bada, Rokaya Bada, Damai Bada, Bhandari-Lawar, Budha Bohara, Thapa Lawar, Bhandri Thapa, Siyal Bada)
Total area for this crop in the community (total cropped area in parentheses)	120 (258) ha
No. of modern varieties	0
Proportion of farm (%) devoted to growing traditional varieties	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	5
No. of farms in the community (total HHs in parenthesis)	180 (759)
Average no. of variety units per farm (or HH)	1.55
Average evenness or Dominance (Simpson)	0.32
Total units or varieties (richness) in the community	5
Structure –between/within farms % divergence between sites	20.16%
Comments, Caveats	This information was estimated from baseline survey conducted in 1998. Community is defined as a group of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village/farmer group/hamlet–household–farm. We have reported here based on ecosite.
Standard Error, CV	SE, 0.1866, CV, 5.918%
Deeper analysis, basis for the specific analysis: phenotypes, isozymes, microsats, pedigree analysis	Phenotypes Isozymes
Purpose of the study	Varietal genetic distinctiveness and diversity assessment
Number of types (alleles or genotypes) per 'locus' – any measure of richness	Phenotypes: 9 quantitative and 9 qualitative traits Isozymes: 5 isozyme systems (PRX, EST, MDH, PGD and IDH), Avg. number of alleles per locus: 1.38
Divergence between populations	Avg. coefficient of gene differentiation: 0.149
Comments, Caveats	Not all the landraces were studied. We report here only the summary of agromorphological and isozyme analyses. Sample sizes were not consistent. Data are presented by landraces, not sites. Some data are from on farm and some from on station.

**Table 3. Comparative amount and distribution of finger millet diversity on-farm at the *in situ* project in Bara, Kaski and Jumla districts in Nepal, 1998–2003**

	Jumla	Kaski	Bara
Community Varieties	Talium and Kartikswami 18 (Khalla Silam, Gharti-Kami-Damai, Shreedhuska Bahun, Shreedhusak Thar, Bayal Katiya, Sarki Bada Ka, Sarki Bada Kha, Tallo Rokayabada, Dharala Bada, Talium Thar Bada, Lawar Bada, Rokaya Bada, Damai Bada, Bhandari-Lawar, Budha Bohara, Thapa Lawar, Bhandri Thapa, Siyal Bada)	Begnas 22 (Unnatsil, Devasthan, Mahila Sabisa, Upallo Talbeshi, Rupakot Jamalkuna, Rupa Sundaridada, Kholabeshi, Pourakhe, Chaur, Kalimati, Archalthar, Viveksil Amasamu, Rupa Srijana Ama, Punithar, Poudelthar, Aduwabari, Simalpata, Kotbari, Adhikarithar, Majthar, Dandathar, Bisaunathar)	Kachorwa 22 (Pachiyari Math tol, Mahabir tol, Sarkari Inar tol, Baba Tol, Bording tol, Bajar tol, Math tol, Baluwa tol, Nadi tol, Dhanbhakhari tol, Sadak tol, Chauki tol, Masjid tol, Gola tol, Bhakta Inar tol, Budhuwa Inar tol, Ramana tol, Kamiti tol, Bajarangi tol, Parasnath tol, Purohit tol, Office tol)
Total area (ha) for this crop in the community (total cropped area in parentheses)	35 (258)	133 (363)	0.7 (627)
No. of modern varieties	0	0	0
Proportion of farm (%) devoted to growing traditional varieties	100	100	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	12	24	6
No. of farms in the community (total HHs in parenthesis)	180 (759)	206 (941)	202 (914)
Avg. no. of variety units per farm (or HH)	1.311	1.22	0.094
Avg. evenness or Dominance (Simpson)	0.18	0.17	0.002
Total units or varieties (richness) in the community	12	24	6
Structure – between/within farms % divergence between sites	70.2%	78.9%	99.7%
Comments, Caveats	This information was derived from baseline survey conducted in 1998. Community is defined as a group of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village–household–farm. We have reported here based on ecosite. Most of the information was collected from published documents. CV was estimated with SE (due to 0 values in many cases). This information is drawn from 180, 206 and 202 households of Talium, Begnas and Kachorwa, respectively. In some cases landrace areas were missing and some households had no landraces. Area estimation of cucumber and sponge gourd were not feasible. All data except results of deeper analysis were from baseline survey.		
Standard Error, CV	SE, 0.0045, CV 0.413%	SE, 0.201, CV, 7.16%	SE, 0.0034, CV, 3.45%

**Buckwheat (*Fagopyrum esculentum* L. and *Fagopyrum tataricum* L.)**

Common (*F. esculentum*) and bitter (*F. tataricum*) buckwheat are commonly grown in high mountaineous regions of Nepal. Buckwheat is basically a minor crop in Nepal; it is an important crop in high hills but is also grown in middle hills and inner *terai*. We studied this crop in 18 hamlets of Jumla districts during 1998–2000 (Table 4). We recorded a total of 10 ha of land under buckwheat cultivation by 180 households. Farmers cultivated 6 local landraces with 0.32 landraces/HH in the studied area. The Simpson index of this crop (0.03) indicated that landraces are evenly distributed in the community. This was evident from higher

divergence (97.2%) for buckwheat cultivation recorded between farms in the study sites. We conducted deeper analysis of diversity for buckwheat landraces using 3 isozymes (Table 4) and recorded higher alleles per locus (1.96) and an average coefficient of genes differentiation of 0.227, indicating higher genetic diversity.

**Table 4. Comparative amount and distribution of buckwheat diversity on-farm at the *in situ* project in Jumla district in Nepal, 1998–2000.**

Jumla	
Community Varieties	Talium and Kartikswami 18 (Khalla Silam, Gharti-Kami-Damai, Shreedhuska Bahun, Shreedhusak Thar, Bayal Katiya, Sarki Bada Ka, Sarki Bada Kha, Tallo Rokayabada, Dharala Bada, Talium Thar Bada, Lawar Bada, Rokaya Bada, Damai Bada, Bhandari-Lawar, Budha Bohara, Thapa Lawar, Bhandri Thapa, Siyal Bada)
Total area (ha) for this crop in the community (total cropped area in parentheses)	10 (258)
No. of modern varieties	0
Proportion of farm (%) devoted to growing traditional varieties	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	6
No. of farms in the community (total HHs in parentheses)	180 (759)
Avg. no. of variety units per farm (or HH)	0.32
Avg. evenness or Dominance (Simpson)	0.03
Total units or varieties (richness) in the community	6
Structure – between/within farms % divergence between sites	97.2%
Comments, Caveats	This information was estimated from baseline survey conducted in 1998. Community is defined as a group of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village/farmer group/hamlet–household-farm. We have reported here based on ecosite.
Standard Error, CV	SE, 0.0038, CV, 1.47%
Deeper analysis: basis for the specific analysis (phenotypes, isozymes, microsats, pedigree analysis)	Phenotypes Isozymes
Purpose of the study	Varietal genetic distinctiveness, Diversity assessment
Number of types (alleles or genotypes) per 'locus' – any measure of richness	Phenotypes: 4 quantitative and 10 qualitative traits Isozymes: 3 isozyme systems (PRX, MDH and ADH), Avg.number of alleles per locus: 1.96
Divergence between populations	Avg. coefficient of gene differentiation: 0.227
Comments, Caveats	Not all the landraces were studied. We report here only the summary of agromorphological and microsatellite analyses. Sample sizes were not consistent. Data are presented landrace-wise. Some data are from on-farm and some from on-station. Most of the information is from published documents.

### **Taro (*Colocasia esculenta* L.)**

Taro is an important root crop in home gardens and arable fields in Nepal. It was found to be grown from the low-lying Bara site (n=3 varieties) to the high mountain site Jumla (n=1 variety); however, the greatest richness of taro diversity was found in the mid-hill site of Begnas (Rijal *et al.* 2003). The diversity data of taro is based on 22 hamlets of Begnas area in Kaski district (Table 5). We found that 70% of households cultivated different taro landraces in 363 ha of land in the studied area. During a baseline survey in 1998, we recorded 24

landraces of taro and none of the improved varieties in the project site. We estimated 1.52 landraces/HH in Begnas area, indicating farmers grow different landraces of taro at household level. The deeper analysis of dominance using Simpson index revealed that the dominance of particular taro landraces was less, resulting in a relatively even distribution of taro landraces. This was evident from the divergence (77.2%) of taro landraces between farms in the community. The molecular analysis of taro landraces using 6 isozymes conducted by the project revealed 1.96 alleles/locus. Given the information on diversity index and molecular analysis, we conclude that Begnas area has higher diversity of taro.

**Table 5. Comparative amount and distribution of taro diversity on-farm at the *in situ* project in Kaski district in Nepal, 1998.**

	<b>Kaski</b>
Community	Kaski (Begnas)
Varieties	22 (Unnatsil, Devisthan, Mahila Sabisa, Upallo Talbeshi, Rupakot Jamalkuna, Rupa Sundaridada, Kholabeshi, Pourakhe, Chaur, Kalimati, Archalthar, Viveksil Amasamu, Rupa Srijana Ama, Punithar, Poudelthar, Aduwabari, Simalpata, Kotbari, Adhikarithar, Majthar, Dandathar, Bisaunathar)
Total area (ha) for this crop in the community (total cropped area in parentheses)	70% HH (363)
No. of modern varieties	0
Proportion of farm (%) devoted to growing traditional varieties	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	24
No. of farms in the community (total HHs in parentheses)	206 (941)
Avg. no. of variety units per farm (or HH)	1.524
Avg. evenness or Dominance (Simpson)	0.20
Total units or varieties (richness) in the community	24
Structure – between/within farms % divergence between sites	77.2%
Comments, Caveats	This information was derived from baseline survey conducted in 1998. Community is defined as a group of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village/farmer group/hamlet–household–farm. We have reported here based on ecosite.
Standard Error, CV	SE, 0.0022, CV, 1.325%
Deeper analysis: basis for the specific analysis (phenotypes, isozymes, microsats, pedigree analysis)	Phenotypes
Purpose of the study	Isozymes
Number of types (alleles or genotypes) per 'locus' – any measure of richness	Diversity assessment
Comments, Caveats	Phenotypes: 8 quantitative and 10 qualitative traits Isozymes: 6 isozyme systems (PRX, EST, ACP, PGD, MDH and IDH), Avg. number of alleles per locus: 1.96 Not all the landraces were studied. We report here only the summary of agromorphological analyses. Sample sizes were not consistent. Data are presented landrace-wise. Diversity indices based on isozymes were not estimated.



### Cucumber (*Cucumis sativus* L.)

Cucumber is grown on a small scale in home gardens of *terai*, mid-hills and high hills in Nepal. This indicates that this crop has high diversity adapted to diverse agroecozones in Nepal. We therefore studied diversity of this crop in all three project sites in Bara, Kaski and Jumla. In Kaski, 85% of households cultivated this crop followed by 52.8% in Jumla but few households (3.46%) grew this crop in Bara (Table 6). The number of cultivated landraces was also few, only 4 in Bara site compared with 15 in Kaski and 10 in Jumla. However, the proportion of area for landraces versus modern varieties was only 42% in Kaski but it was 100% in Jumla and Bara. The average landraces/HH was also higher in Kaski (0.78) followed by 0.58 in Jumla and 0.035 in Bara. The Simpson index estimated for each study site revealed that there was relatively less dominance of particular landraces which was evident from a higher divergence percentage between farms in each project site (Table 6).

**Table 6. Comparative amount and distribution of cucumber diversity on-farm at the *in situ* project in Bara, Kaski and Jumla districts in Nepal, 1998.**

	Jumla	Kaski	Bara
Community Varieties	Talium and Kartikswami 18 (Khalla Silam, Gharti-Kami-Damai, Shreedhuska Bahun, Shreedhusak Thar, Bayal Katiya, Sarki Bada Ka, Sarki Bada Kha, Tallo Rokayabada, Dharala Bada, Talium Thar Bada, Lawar Bada, Rokaya Bada, Damai Bada, Bhandari-Lawar, Budha Bohara, Thapa Lawar, Bhandri Thapa, Siyal Bada)	Begnas 22 (Unnatsil, Devasthan, Mahila Sabisa, Upallo Talbeshi, Rupakot Jamalkuna, Rupa Sundaridada, Kholabeshi, Pourakhe, Chaur, Kalimati, Archalthar, Viveksil Amasamu, Rupa Srijana Ama, Punithar, Poudelthar, Aduwabari, Simalpata, Kotbari, Adhikarithar, Majthar, Dandathar, Bisaunathar)	Kachorwa 22 (Pachiyari Math tol, Mahabir tol, Sarkari Inar tol, Baba Tol, Bording tol, Bajar tol, Math tol, Baluwa tol, Nadi tol, Dhanbhakhari tol, Sadak tol, Chauki tol, Masjid tol, Gola tol, Bhakta Inar tol, Budhuwa Inar tol, Ramana tol, Kamiti tol, Bajarangi tol, Parasnath tol, Purohit tol, Office tol)
Total area (ha) for this crop in the community (total cropped area in parentheses)	52.8% HH (258)	85% HH (363)	3.46% HH (627)
No. of modern varieties	0	1	0
Proportion of farm (%) devoted to growing traditional varieties	100	42	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	10	15	4
No. of farms in the community (total HHs in parenthesis)	180 (759)	206 (941)	202 (914)
Avg. no. of variety units per farm (or HH)	0.583 (2-5 vines /HH)	0.782 (2-3 vines/HH)	0.035 (2-3 vines/HH)
Avg. evenness or Dominance (Simpson)	0.036	0.056	0.00
Total units or varieties (richness) in the community	10	15	4
Structure – between/within farms, % divergence between sites	95.9%	93.4%	100%
Comments, Caveats	This information is from baseline survey. Community is groups of households located geographically close, but their farm may be in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village/farmer group/hamlet–household–farm. We have reported here based on ecosite.		
Standard Error, CV	SE, 0.2044, CV, 16%	SE, 1.1705, CV, 35.88%	SE, 0.159, CV, 44.601%

**Sponge Gourd (*Luffa cylindrica* L.)**

Sponge gourd is a popular summer crop vegetable cooked in every Nepalese kitchen, especially in mid-hills and *terai*. We studied the diversity of this crop in Kaski and Bara districts during 1998 to 2003 (Table 7). We found that more than 91% in Kaski and 63% of households in Bara cultivated this crop on-farm. It was interesting to note that all of the 15 and 16 varieties, respectively, grown by farmers were local landraces. We found the average number of varieties/HH higher in Kaski (1.48) than in Bara (0.76). It was extremely interesting to note that there was less dominance of particular landraces in Bara than in Kaski. This was evident from a low Simpson index for Bara (0.037) and higher divergence between farms (95.9%) compared with Kaski (Table 7).

**Table 7. Comparative amount and distribution of sponge gourd diversity on-farm at the *in situ* project in Bara and Kaski districts in Nepal, 1998–2003.**

	<b>Kaski</b>	<b>Bara</b>
Community	Begnas	Kachorwa
Varieties	22 (Unnatsil, Devasthan, Mahila Sabisa, Upallo Talbeshi, Rupakot Jamalkuna, Rupa Sundaridada, Kholabeshi, Pourakhe, Chaur, Kalimati, Archalthar, Viveksil Amasamu, Rupa Srijana Ama, Punithar, Poudelthar, Aduwabari, Simalpata, Kotbari, Adhikarithar, Majthar, Dandathar, Bisaunathar)	22 (Pachiyari Math tol, Mahabir tol, Sarkari Inar tol, Baba Tol, Bording tol, Bajar tol, Math tol, Baluwa tol, Nadi tol, Dhanbhakhari tol, Sadak tol, Chauki tol, Masjid tol, Gola tol, Bhakta Inar tol, Budhuwa Inar tol, Ramana tol, Kamiti tol, Bajarangi tol, Parasnath tol, Purohit tol, Office tol)
Total area (ha) for this crop in the community (total area in parentheses)	91.3% HH (363)	66.3% HH (627)
No. of modern varieties	0	0
Proportion of farm (%) devoted to growing traditional varieties	100	100
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	15	16
No. of farms in the community (total HHs in parenthesis)	206 (941)	202 (914)
Avg. no. of variety units per farm (or HH)	1.48	0.762
Avg. evenness or Dominance (Simpson) <sup>7</sup>	0.251	0.037
Total units or varieties (richness) in the community	15	16
Structure – between/within farms, % divergence between sites	64.4%	95.9%
Comments, Caveats	This information was collected from a baseline survey conducted in 1998. Community is defined as a group of households located geographically close, but their farm may be scattered in other locations or communities. Hierarchical structure of ecosite: ecosite–community–village–household–farm. We have reported here based on ecosite. Most of the information is from published documents.	
Standard Error, CV	SE, 0.1581, CV, 4.9	SE, 0.3945, CV, 12.148%

**Pigeon pea (*Cajanus cajan* L.)**

Pigeon pea is a leguminous crop of *terai* and inner *terai* communities and farmers mostly grow this crop on rice bunds in these areas. Therefore we studied the diversity of this in Bara project site during 1998–2003 (Table 8). The diversity data of this crop represent the 22 hamlets of Kachorwa community. We found that 27.2% of survey households cultivated this crop in 4.26 ha. We recorded 5 varieties of pigeon pea, all of them landraces. The average number of landraces per farm was recorded as 0.28. The Simpson index was estimated as 0.005, indicating a higher even distribution of landraces in the community which was

evident from higher divergence (99.4%) between farming households. This revealed that the reported five landraces were evenly distributed in the community. We also conducted deeper analysis of diversity at gene level using 4 isozymes. The molecular study revealed that the number of alleles per locus was 1.93 and average coefficient of gene differentiation was 0.265 in Bara ecosite, indicating higher genetic diversity in reported landraces.

**Table 8. Comparative amount and distribution of pigeon pea diversity on-farm at the *in situ* project in Bara district in Nepal, 1998–2003.**

	<b>Bara</b>
Community	Kachorwa
Varieties	22 (Pachiyari Math tol, Mahabir tol, Sarkari Inar tol, Baba Tol, Bording tol, Bajar tol, Math tol, Baluwa tol, Nadi tol, Dhanbhakhari tol, Sadak tol, Chauki tol, Masjid tol, Gola tol, Bhakta Inar tol, Budhuwa Inar tol, Ramana tol, Kamiti tol, Bajarangi tol, Parasnath tol, Purohit tol, Office tol)
Total area (ha) for this crop in the community (total area in parentheses)	27.2% HH, 4.26 (627)
No. of modern varieties	0
Proportion of farm devoted to growing traditional varieties	100%
Basic countable recognizable units – LR, Use-type, Morph-type, Land-type	5
No. of farms in the community (total HHs in parenthesis)	202 (914)
Average no. of variety units per farm (or HH)	0.282
Average evenness or Dominance (Simpson)	0.005
Total units or varieties (richness) in the community	5
Structure – between/within farms, % divergence between sites	99.4%
Comments, Caveats	This information is from baseline survey. Community is groups of households located geographically close, but their farm may be in other location or community. Hierarchical structure of ecosite: ecosite–community–village–household–farm. We have reported here based on ecosite.
Standard Error, CV	SE, 0.1904, CV, 30.13%
Deeper analysis: basis for the specific analysis (phenotypes, isozymes, microsats, pedigree analysis)	Phenotypes
Purpose of the study	Isozymes
	Varietal genetic distinctiveness
	Diversity assessment
Number of types (alleles or genotypes) per 'locus' – any measure of richness	Phenotypes: 9 quantitative and 10 qualitative traits Isozymes: 4 isozyme systems (IDH, PRX, MDH and ADH), Avg. number of alleles per locus: 1.931
Divergence between populations	Avg. coefficient of gene differentiation: 0.2683
Comments, Caveats	Not all the landraces were studied. We report here only the summary of agromorphological and isozymes analyses. Sample sizes were not consistent. Data are presented landrace-wise. Some data are from on-farm and some from on-station. Most of the information is from published documents.

## Conclusions

The diversity of crop varieties has been maintained by farming communities on-farm over the generations. Studies carried out to understand the amount and distribution of local crop diversity of eight mandate crops in Nepal revealed that farmers continue to maintain and manage substantial diversity in agricultural production systems. Traditional crop varieties, mostly of neglected and minor crops, are an important component of this diversity and constitute a key biological asset that supports livelihoods of poor farmers in difficult production environments. We have made an attempt to understand the diversity and summarized the basic diversity data on eight mandate crops in this paper; this will serve as

an important document to monitor the varietal dynamics over time. We have seen great variations of crop genetic resources studied in Bara, Kaski and Jumla project sites. This information furnishes important references for future research on *in situ* conservation of agrobiodiversity on-farm. This paper also demonstrates the various methods employed to summarize basic diversity data in other plant genetic resources in future. The challenges of the studies are how farmers and researchers could work together to harvest the benefits of available local diversity for the welfare of local farmers and communities.

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## Four-cell analysis as a decision-making tool for conservation of agrobiodiversity on-farm

Ram B. Rana, Bhuwon R. Sthapit, C. Garforth, Anil Subedi and Devra I. Jarvis

### Abstract

Understanding the amount and distribution of local crop diversity at the community level is basic information required for managing agrobiodiversity on-farm. There is a lack of participatory methodologies that help researchers and farmers to understand distribution patterns of local crop diversity and the rationale for such distribution. This paper explores 'four-cell analysis' as a decision-making tool for assisting agrobiodiversity conservation decisions. Rice (*Oryza sativa* L.) varieties are arranged into four cells on the basis of area planted and number of households growing the variety. Analysis of use value of varieties in Begnas (mid-hill) and Kachorwa (plain) sites in Nepal suggests varieties have food security, market sale, niche adaptation, sociocultural and medicinal values. Landraces and MVs (modern varieties) are cultivated for food security reasons in a large area by many households. Aromatic fine type rice landraces grown for selling in the market also fall in this category. Limited landraces and MVs have specific adaptation and they are grown by few households in a relatively large area. Socioculturally important landraces are grown by many households but in a small area. Landraces with no apparent use values are grown by few households in a small area. Use of a four-cell analysis technique to decide on different value-addition options such as repatriation, participatory plant breeding, agronomic research, awareness creation and market promotion is highlighted for landraces falling in different use categories. The analysis suggests that landraces with no apparent use value to farmers are unlikely to be conserved on-farm, whereas landraces with some use value to farmers can be conserved on-farm to varying degrees.

**Key words:** Agrobiodiversity conservation, use value, four-cell analysis, participatory plant breeding, rice, Nepal

### Introduction

Three different types of values of crop varieties can be distinguished: direct, indirect and option value. Direct or use value is the simplest and obvious one that refers to harvest and uses of crop varieties. Indirect values refer to the environmental services or ecological health to which the crop varieties contribute, but farmers may not observe or notice the relationship. Option values refer to future use of crop varieties (Brush 2000). Socioeconomic and cultural (food security, market, religious and cultural uses) and adaptive traits, which jointly represent 'use value' of variety (landraces and MVs), determine the existence of these varieties on-farm. Farmers value certain aspects in the varieties—either socioeconomic or adaptive traits or both—and the comparative advantages of their preference directly determine the area coverage and the number of households (HHs) cultivating these varieties at community level (Rana 2004).

Studies have asserted the need to understand the inter-connectedness of cultural diversity and agrobiodiversity, including how farmers incorporate new crops and varieties in their current repertoire to meet cultural and environmental needs of society and its farming systems (Soleri and Cleveland 1993). Other studies have indicated that economic status and food culture/culinary preferences promote diversity on-farm (Brush 1989; Gurung and Vaidya 1998).

To measure the status of crop diversity in the field the most common method is counting named varieties. The number of varieties (richness) and their pattern of distribution in terms of area coverage (evenness) are key indicators for local crop diversity at the landscape level. For farmers, genetic diversity means varietal diversity, which farmers can clearly distinguish on the basis of agromorphological traits,

phenological attributes, postharvest characteristics, and differential adaptive performance under abiotic and biotic stresses (Sthapit and Subedi 1997).

Scientists recognize that “the scientific basis and optimal procedures for on-farm conservation are lacking” (Brown 2000:30). Vernooy (2003) raised the complex issues of agrobiodiversity conservation, and the need to develop methodologies that combine understanding of biological and social aspects. This study therefore attempts to understand how ‘use values’ of rice contribute to varietal diversity on-farm. Rice was selected for this study because of its importance for livelihoods, its wide range of diversity and widespread associated local knowledge. Data on rice varieties from two contrasting study sites in Nepal—Begnash and Kachorwa villages—were arranged into four cells using area coverage and number of households growing the variety as criteria for classification. Then we explored the use values associated with varieties clustered in each cell. By doing so, efforts were made to develop ‘four-cell analysis’ as a decision-making tool for management of agrobiodiversity (*ex situ* or *in situ*) and for deciding value-addition options suitable for varieties falling in different categories.

### Materials and methods

The dataset used in the current study is derived from a socioeconomic and agroecological study conducted in Begnash and Kachorwa ecosystems (Rana 2004). Household (HH) was taken as the sampling unit for the study. The study employed proportionate stratified random sampling design, and samples of 206 and 202 HHs were selected out of 941 and 914 HHs in Begnash and Kachorwa study sites respectively (Table 1). Information was collected on respondent profile, household features, and farm characteristics including access to market and information. Detailed information was elicited on varieties: growing environment and area coverage, preferred (un-preferred) traits, and religious and cultural values, if any, of each variety. Data were analyzed using SPSS Windows.

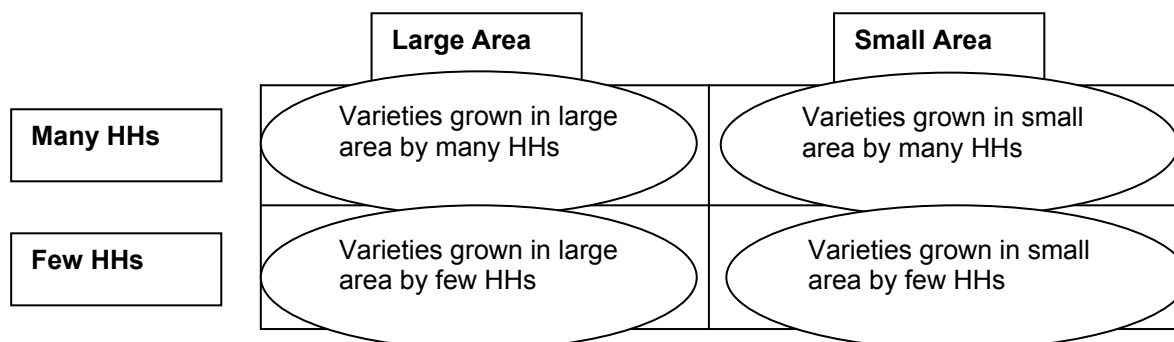
**Table 1. Characteristic features of study sites in Nepal.**

Variables	Unit	Begnash	Kachorwa
Elevation (range)	Metres asl	668-1206	<100
Annual rainfall	mm/year	3979	1515
Mean annual temperature	°C	20.9	24.6
Total number of households	Number	941	914
Sampled households (socioeconomic study)	Number	206	202
Mean family size	Person/HH	6.5±0.2	6.5±0.2
Family members engaged in agriculture	Persons/HH	2.0±0.1 (166) <sup>†</sup>	2.8±0.1 (192)
Family members engaged in non-agriculture activities	Person/HH	1.4±0.2 (14)	1.4±0.1 (96)
Households with female decision-makers in agriculture	Percentage of HHs	58	15
Education status of decision-maker in household	Literacy percentage	56	41
Agriculture as principal occupation	Percentage of HHs	70	97
Mean agricultural land area	Hectare/HH	0.7±0.1 (196)	0.8±0.1 (188)

<sup>†</sup> Figures in parentheses indicate number of responding households.

One of the ways to classify different varieties found in a given geographic location is to arrange them on the basis of number of HHs growing them and the mean area coverage per HH (Figure 1). Number of HHs growing the variety can be classified into two categories: many HHs and few HHs, with an arbitrary cut-off point of 5 HHs. Mean area of varieties could be classified into large area versus small area by identifying the arbitrary cut-off point, which could be achieved by arranging the mean figures of all varieties in either ascending or

descending order and taking a point from where the graph abruptly changes slope. In this case, we have taken an arbitrary unit of 5 HHs as cut-off point for both study sites because the sample sizes are more or less equal. But the mean area per household for different varieties between the sites varies significantly; hence different figures are used. In the case of Begnas, the cut-off point for mean area coverage is 0.05 ha/HH, whereas for Kachorwa the value is 0.2 ha/HH.



**Figure 1. Classification of rice varieties into four cells using area and number of HHs.**

With this technique, we can classify all the varieties grown in a 2x2 matrix, allocating each to one of the four cells: many HHs, large area; many HHs, small area; few HHs, large area; and few HHs, small area. After the deployment of varieties in different cells, in subsequent steps we analyzed the characteristics of varieties that fall within and between different cells. In focus group discussion, we can ask a simple question, “Why is variety A grown in large areas by many HHs in the village and why is variety B grown in small areas by many HHs?”, and record the reasons. This tool encourages farmers to bring out common factors for choosing and allocating plots to a certain variety. We also explored different value-addition options amenable to different landraces to make them more competitive. Finally, the four-cell analysis model helped community as well as researchers and development professionals to understand the threat of genetic erosion, and current knowledge is used to suggest conservation actions on landraces, specifically whether to conserve them on-farm or *ex situ* in genebanks.

## Results and discussion

### Four-cell analysis: classification of varieties into different categories based on area planted and number of households growing them

Using four-cell analysis all the varieties (landraces and MVs) found in the study sites have been deployed into different cells (Table 2, Table 3). Out of 56 varieties in Begnas, 15 (27%) fall in the first cell, i.e. varieties grown by many households in a larger area. Of those 15 varieties, 11 (73%) were landraces and the rest were MVs. Interestingly, of the five MVs in Begnas, four fall in this category suggesting that they were popular with farmers. Varieties in this category play a significant role in household food security, and some landraces such as *Jethobudho* and *Pahale* are grown mainly for selling in the market. Others, including landraces such as *Mansara* and *Kathe Gurdi*, are well adapted to marginal environments and cover a considerable area in Begnas village, 20–28% of the total rice land. In the second category, few HHs growing the variety in a large area, we have 12 (24%) varieties, all landraces. Varieties included in this category have specific adaptation to certain niche environments; examples include *Pakhe Jarneli* and *Naulo Madhise*. *Pakhe Jarneli* is fine type rice with good eating quality that does well under rain-fed conditions. *Naulo Madhise* is early maturing with better yield and eating quality than *Madhise*.



**Table 2. Classification of varieties<sup>†</sup> into different cells using four-cell analysis at Begnas.**

No. of HHs	Large area	Small area
Many households	Ekle (85, 0.16) <sup>‡</sup> , <b>Mansuli</b> (59, 0.2), Thulo Madhise (53, 0.17), Kathe Gurdi (47, 0.1), Mansara (44, 0.12), <b>Radha7</b> (37, 0.15), Thulo Gurdi (33, 0.13), Jethobudho (30, 0.11), Pafele (23, 0.14), Jhinuwa Ghaiya (23, 0.07), <b>Radha9</b> (12, 0.15), <b>CH45</b> (13, 0.12), Lahere Gurdi (12, 0.17), Rato Ghaiya (8, 0.1), Sano Madhise (6, 0.23): landraces = 11; MVs = 4	Rato Anadi (71, 0.02), Seto Anadi (56, 0.02), Dhabe Jarneli (19, 0.02), Seto Bayarni (12, 0.03), Aanga (9, 0.04), Tunde (7, 0.04): landraces = 6; MVs = 0
Few households	Seto Ghaiya (2, 0.08), Naulo Madhise (3, 0.18), Jiri Ghiya (3, 0.11), Kalo Gurdi (2, 0.26), Jhauri (2, 0.24), Mana Muri (2, 0.09), Pakhe Jarneli (2, 0.08), Kunchali Ghaiya (2, 0.06), Seto Gurdi (1, 0.23), Gauriya (1, 0.15), Jhyali Rato Ghaiya (1, 0.1), Mala (1, 0.08): landraces = 12; MVs = 0	Basmati (5, 0.02), Kalo Jhinuwa (5, 0.01), Bichare Ghaiya (4, 0.05), Gurdi Ghaiya (4, 0.04), Seto Jhinuwa (4, 0.03), Naltume (3, 0.04), Biramphul (2, 0.03), Thapachini (2, 0.01), Lame (1, 0.05), <b>Kanchi Mansuli</b> (1, 0.04), Kanajira Ghaiya (1, 0.03), Katush Ghaiya (1, 0.03), Lahare Ghaiya (1, 0.03), Masino Ghaiya (1, 0.03), Tunde Jhinuwa (1, 0.03), Barmali (1, 0.02), Chobo (1, 0.02), Kalo Bayarni (1, 0.04), Kalo Tunde Jhinuwa (1, 0.02), Rate (1, 0.02), Pakhe Ramani (1, 0.01), Bayarni Jhinuwa (1, 0.03), Jhinuwa Basmati (1, 0.02): landraces = 22; MVs = 1

<sup>†</sup> Varieties highlighted in **bold** indicate modern varieties.<sup>‡</sup> Numbers in parentheses indicate (number of households, mean area in ha per household).**Table 3. Classification of varieties<sup>†</sup> into different cells using four-cell analysis at Kachorwa**

No. of HHs	Large area	Small area
Many households	<b>China4</b> (120, 0.28) <sup>‡</sup> , <b>Masula</b> (102, 0.44), <b>Sabetri</b> (64, 0.37), <b>Chandina</b> (39, 0.21), Mutmur (28, 0.22), Nakhisaro (16, 0.23), <b>Jiri</b> (15, 0.36), <b>Philips</b> (14, 0.24), <b>Mansuli</b> (9, 0.29), <b>Lalkafarm</b> (7, 0.25), <b>Television</b> (6, 0.32): landraces = 2; MVs = 9	Basmati (36, 0.13), Sotwa (12, 0.19), Sathi (8, 0.07): landraces = 3; MVs = 0
Few households	<b>Meghdut</b> (5, 0.23), <b>Natmasula</b> (3, 0.42), <b>Ekathar</b> (3, 0.3), <b>Faram</b> (3, 0.22), <b>Kanchi Mansuli</b> (2, 0.44), Mansara (2, 0.33), <b>Karma</b> (2, 0.28), Aashani (1, 1.35), <b>CH45</b> (1, 0.53), Bhatthi (1, 0.4), <b>Usha</b> (1, 0.27): landraces = 3; MVs = 8	Sokan (4, 0.07), Sarho (3, 0.18), <b>Jaya</b> (3, 0.17), Lajhi (3, 0.13), <b>Sona Mansuli</b> (2, 0.17), <b>Radha5</b> (2, 0.1), <b>Rango</b> (2, 0.09), <b>Mallika</b> (1, 0.2), Dudhraj (1, 0.2), <b>Batsar</b> (1, 0.2), Satraj (1, 0.017), Lalkakartika (1, 0.17), <b>Kataush</b> (1, 0.15), <b>Ratrani</b> (1, 0.12), <b>Dipahi</b> (1, 0.12), <b>Gajargoul</b> (1, 0.1), Khera (1, 0.1), <b>Laltengar</b> (1, 0.07), Aanga (1, 0.07), Dudhi Saro (1, 0.05), <b>Ghutani</b> (1, 0.03), Anadi (1, 0.03): landraces = 10; MVs = 12

<sup>†</sup> Varieties highlighted in **bold** indicate modern varieties.<sup>‡</sup> Numbers in parentheses indicate (number of households, mean area in ha per household).

A third category includes varieties grown by many HHs but in small areas. Not many landraces are included in this category and certainly not the MVs because mostly the landraces with sociocultural, religious and medicinal values are included in this category. A total of six (11%) different landraces fall in this category. If we combine *Rato Anadi* and *Seto Anadi* and consider them as one group then *Anadi* is the most widely grown landrace in the area but grown in a very small area (0.02 ha/HH) as they are grown for specific uses. *Anadi* is the only glutinous rice found in Nepal and is used for making *Latte*<sup>1</sup>, *Khatte*<sup>2</sup> and *Siraula*<sup>3</sup>.

<sup>1</sup> *Latte* is prepared by soaking rice (de-husked) for about 12–24 hours then cooking it in ghee or oil and sugar is added while continuously stirring. Unlike normal cooking of rice, no water is added while cooking *Latte*. It is mainly consumed during 'Saune Sakranti' – a festival celebrated in the

Farmers believe it possesses some medicinal values as well. *Aanga* is specifically grown in most marginal environments and considered to have medicinal value.

Finally, the fourth category represents varieties grown by few HHs in small areas, and there are numerous varieties falling in this category—22 landraces and one MV. Varieties clustered in this group do not seem to have specific characters; rather, different landraces and MVs have different use values but these varieties do have better-performing competitors, and hence they have been forced out to the fringe. It is likely that newly introduced landraces or MVs could also fall in this category in the process of testing by farmers because they would be tested by few HHs in small areas.

Results from Kachorwa suggest that, out of 47 varieties 11 (23%) are grown by many households in large areas that contribute considerably to household food security. Of those 11 varieties only 2 are landraces and rest are MVs. The landraces falling in this category are *Mutmur* and *Nakhisaro* and they are both adapted to water-stressed conditions. In the second category, i.e. few HHs, large areas, there are 11 (23%) varieties with 3 landraces and 8 MVs. These varieties do play an important role in food security of specific households. Varieties with specific adaptation fall in this category, examples include *Bhathi*, which is a deep-water (floating) rice adapted to swampy land and on edges of ponds. Some newly introduced MVs also fall within this category: examples include *Ekathar* and *Natmasula*, which are yet to be widely adopted.

In the third category (many HHs, small area) we have what have been categorized as socioculturally and religiously important landraces. In Kachorwa as well there is no MV in this category and the number of landraces is only three. Basmati is highly valued for its good eating quality (soft and aromatic when cooked) and its consumption is associated with social prestige in community. Better-off households consume Basmati on a regular basis whereas poor households consume it on special occasions and offer it to guests in the house. *Sathi* rice is a must in *Chathi* festival, celebrated in *terai* (southern plain) regions of the country, for making offerings to *Chathi Maiya* the Hindu goddess, and for preparing a variety of sweets and dishes during the festival. Finally, in the fourth category (few HHs, small area) there are 22 (47%) varieties, of which 10 are landraces and 12 are MVs. Some of the landraces such as *Lajhi* and *Khera* are reported to have very good eating quality but owing to a lodging problem their yield level is very low, and they are forced out of competition. For the other landraces falling in this category, lack of special traits resulted in their gradual displacement over time. MVs appearing in this category would inevitably be replaced by better-performing MVs.

#### Four-cell analysis: decision-making tool for choosing value-addition options

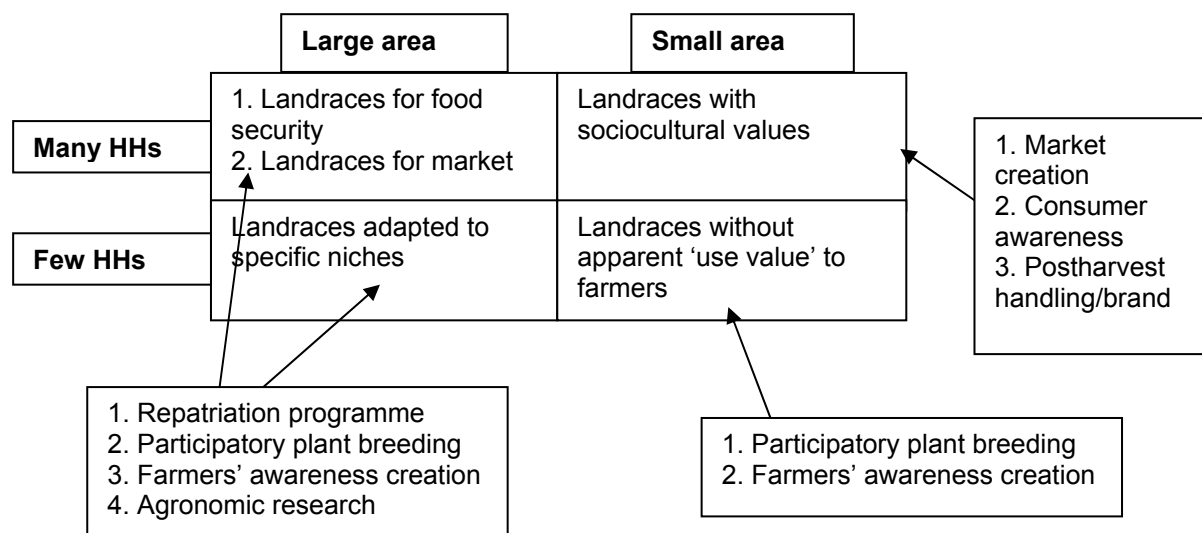
In subsequent paragraphs we analyze the strength of use value in conservation of different types of landraces on-farm as well as suggest value-addition options to rice landraces falling in different categories (Figure 2). The analysis allows us to understand: which landraces have a chance to be promoted as such; which landraces need technical manipulation through plant breeding; which could be promoted by capitalizing on religious and cultural values; still others might have specific adaptive values.

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month of July and '*Pandra Poush*' – festival celebrated in the month of December (Rana *et al.* 2000).

<sup>2</sup> *Khatte* is prepared by light soaking of rice (de-husked) in water then roasting, and is consumed as a snack.

<sup>3</sup> *Siraula* is prepared by soaking husked rice then roasting it until the grains pop; they are left to cool followed by de-husking by either a huller or a paddle pounder. *Siraula* is consumed as a snack mixed with milk to add taste.



**Figure 2. Different value-addition options for landraces falling in different categories.**

The first category of landraces includes those cultivated for food security reasons. These landraces are primarily valued for their productivity. These landraces, though grown by many households in a larger area, are quite vulnerable to genetic erosion because most MVs generated by the national breeding programmes fall within this category. By definition MVs are bred for high yield so landraces may lose out in competition to MVs in the more favourable ecosystems. In Kachorwa study site, MVs already dominate in this role and a similar situation might happen in Begnas if national breeding programmes focused their programmes on the mid-hills. For landraces in this category as well, one of the best options would be to utilize the promising landraces in Participatory Plant Breeding (PPB) programmes as parents and improve their negative traits and make them competitive. In Nepal, the *in situ* project has successfully tested this approach as a way of on-farm management of local crop diversity (Sthapit *et al.* 2002a). Repatriation of these landraces to similar ecosystems in other parts of the country would increase their chances of survival on-farm. Research on finding out appropriate agronomic practices for landraces would make them more competitive with MVs. Awareness creation among the farmers regarding the importance of landraces might increase their chance of survival on-farm.

The second category includes landraces with high value aromatic rice such as *Jethobudho*, *Pahela* and *Basmati*, which are already established as 'brand names' in local and national markets. Thus many HHs in a large area grow these landraces. These landraces have the maximum potential for conservation on-farm, while at the same time contributing to household income because they fetch a premium price in the market. Awareness of producers on the value of supplying quality rice in the market, and market expansion would ensure conservation of these landraces on-farm.

Landraces specifically adapted to niche environments comprise a third category. These landraces serve a specific purpose and are vulnerable to loss because of the change in production environments such as fertility and moisture regimes, and land tenure system. A shift in land ownership resulted in discontinuity of growing deepwater rice for a year in Kachorwa ecosite. Still, these landraces have fair chances of survival on-farm because national breeding programmes do not specifically target these niche environments. Any material generated from the national system performing well under the niche environment is only a coincidence rather than the result of a concerted effort of the breeding programme. In fact, participatory plant breeding (PPB) can add value to some of these landraces that would otherwise be lost from the system (Sthapit *et al.* 2002a, 2002b).

The fourth category of landraces includes culturally important landraces. Their main characteristic features are: they are few in number but many HHs cultivate them in small areas. Because of their important role in religious and cultural ceremonies, many HHs tend to grow them rather than to ask neighbours for grains. These landraces have fair chances of survival on-farm so long as local culture thrives in the community. Increasing the market demand through urban consumers for these culturally important landraces might be an incentive for farmers to cultivate these landraces in larger areas and contribute to HH income. A public awareness campaign on the link between values of landraces diversity and cultural diversity would increase consumer awareness followed by increased demand for the product (Rijal *et al.* 2000).

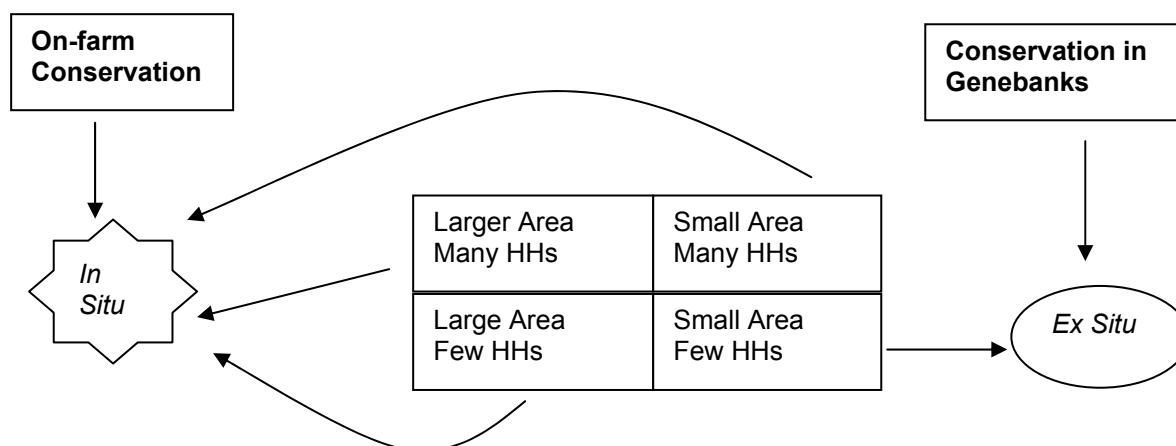
Finally, what we have is a collection of a large number of landraces, which do not seem to have an apparent high use values to farmers. Hence, only a few households in small areas in a community maintain them. Landraces falling in this category would be the most difficult to conserve on-farm as farmers do not have any obvious incentive for conservation. There is still the need to analyze these landraces individually and note the positive and negative traits for their suitability to be used as parents in PPB programmes. Genetically these rare types are reported to be more diverse (*see* Bajracharya *et al.*, Molecular marker diversity in rice landraces of Nepal, this volume). Alternatively, these landraces need to be conserved *ex situ* in a genebank.

#### **Four-cell analysis: identification of common and rare alleles for conservation actions**

This method was also found useful to identify locally common and rare populations at the community level. Marshall and Brown (1975) identified two critical population parameters: (1) the extent of genetic divergence among population, and (2) the level of genetic variation of a population. The basis of describing divergence among populations is frequency and distribution of alleles, leading to four different types of alleles (Figure 1). Populations with locally common alleles are primary targets for collection and conservation. Common, widespread alleles are likely to be found wherever a crop is grown and rare alleles are hard to capture given the limits of collecting missions. The classification of crop landraces used by the four-cell method is very similar to the concept of Marshall and Brown (1975). Communities can use this information to decide the list of locally common cultivars that can be maintained *in situ* and rare types that need collection for genebank or other public awareness purpose.

#### **Four-cell analysis: decision-making tool for agrobiodiversity conservation actions**

The four-cell analysis can be a useful monitoring and decision-making tool because of its ability to capture the dynamics of on-farm management (temporal diversity) when data are gathered at different time scales, based on which we can make decisions about which variety could be conserved on-farm and which variety should go to a genebank for *ex situ* conservation (Figure 3). At the same time, mitigation measures could be developed in partnership with the farming communities to rescue some of the genetic materials (discussed in preceding section). Likewise, the analysis could be used to compare varietal diversity on a spatial dimension, which would allow us to target our resources to programmes in high-priority areas (hot spots of landraces diversity).



**Figure 3. Conservation decision based on four-cell analysis.**

Outputs from four-cell analysis could be an important basis for sound programme planning regarding on-farm conservation initiatives at grassroots level. After varieties are deployed in different cells, participatory sharing of results and further discussion for reasons/explanation for the varieties would enrich the analysis with information on the underlying dynamics. The information needs to be linked with seed production and supply systems, and local-level seed banks would need this kind of information to make decisions on varieties for which the seed needs to be multiplied and which varieties need to be conserved. Links with PPB are equally important. For instance, farmers were reluctant to include the landraces falling in 'small area, many HH group' to be used in PPB programme because they believed PPB would alter the variety and render it useless for religious and cultural ceremonies, for example *Sathi*. Farmers commented that these landraces would continually be grown due to embedded sociocultural, religious and medicinal values (Rana *et al.* 2000). The analysis also shows the importance of complementary conservation strategies in order to conserve diversity of genetic materials. Relying on either of the conservation methods (*ex situ* or *in situ*) would be incomplete in conservation efforts.

### Conclusion

The study revealed that rice landraces grown for food security reasons are vulnerable to genetic erosion because of stiff competition from MVs. Aromatic and fine type landraces have immense potential to contribute to HH cash income, thus they are more likely to survive over time. Socioculturally important landraces, though few in number, are grown by many HHs in very small areas mainly to meet their own purpose. This category of landraces could be conserved on-farm provided demand for them from the urban consumers increases through consumer awareness and market promotion activities. Landraces with niche adaptation are also threatened with erosion because of shifts in farmers' management systems over time. Conservation of landraces on-farm would be rather difficult for those which do not have apparent use value to farmers.

While the incentives for farmers to maintain several landraces in Begnas ecosite exist because of agroecosystem diversity it is less so in the Kachorwa ecosite. Even in Begnas, with the formal system developing new MVs suitable for mid-hill conditions, there are no guarantee that farmers will remain interested in maintaining landrace diversity on-farm. With farmers' aspirations for economic and social change these landraces have to be made more competitive through technical and/or market promotion means so that they contribute to household income. Therefore, it is important to explore policy options and/or technical interventions that might support them. One aspect is clear: complementary conservation

strategies have to be adopted for conserving different landraces on-farm, where the role of *ex situ* conservation cannot be ignored for those landraces that do not appeal to farmers.

Four-cell analysis has successfully been used in discriminating landraces based on their 'use value'. The method has immense potential to be used as a monitoring and decision-making tool for making conservation decisions (*ex situ* or *in situ*) on landraces. However, wider testing of this method is recommended on a range of crops and in different geographic and cultural settings to increase the robustness of the tool before wide-scale application.

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## Genetic variation within and among rice (*Oryza sativa* L.) landraces, varieties and bulks developed by PPB by use of SSR markers

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### Abstract

Rice (*Oryza sativa* L.) is the most intensively evaluated and one of most polymorphic cereal crop species with extensive intra- and intervarietal differentiations. In Nepal, in traditional rice farming systems many diverse landraces are grown in all of the rice agroecosystems from low to high altitude. Landraces are heterogeneous with a blend of different individual plants maintained by farmers in a local environment and carry the traits that are desirable to farmers. An innovative approach of participatory plant breeding (PPB) adopted in Nepal has been successful in developing improved rice varieties and bulks of rice populations with a resulting increase of genetic diversity on-farm. Microsatellite (Simple Sequence Repeats, SSR) markers were used in the present study for the genetic analysis to compare variations in 20 rice varieties: 8 landraces from three major agroecosystems, 3 PPB varieties for mid and high altitudes, and 5 bulk populations selected by farmers in Chitwan and 4 elite varieties used as parents in PPB. The 25 markers under study represented the whole rice genome and revealed 109 alleles with 0–80% of polymorphic alleles (PPA). Landraces represented a genetically diverse group and had multiple alleles at many of the SSR loci studied. PPB bulk conserved as much diversity as found in landraces. The level and distribution of diversity varied with the type of varieties and mode of variety development.

**Key words:** Landraces, participatory plant breeding, simple sequence repeats, polymorphism, heterogeneity

### Introduction

Rice is the most intensively evaluated and one of most polymorphic cereal crop species with the largest *ex situ* germplasm in the world (Virk *et al.* 1996). The genus has extensive intraspecific variation, differentiation into subspecies (*sativa* and *japonica*) and further differentiation into different cultivar groups as landraces, breeding lines, and modern varieties. Landraces are most often heterogeneous with a blend of different individual plants maintained by farmers in a local environment and constitute a significant portion of the cultivated rice gene pool in Asia (Yang *et al.* 1994). Participatory plant breeding (PPB) and participatory variety selection (PVS) are two participatory crop improvement approaches that aim to use, enhance and improve these landraces according to the needs and interest of farmers. These have helped to increase the genetic diversity in farmers' fields (Sthapit *et al.* 1996; Witcombe *et al.* 1996, 2001). Both landraces and varieties produced from PPB are therefore important means of maintaining genetic variation in farmers' fields. In the present study the extent of diversity in landraces and PPB varieties were compared at the molecular level.

For landraces, genetic variability is maintained not only between but also within accessions. Molecular markers, along with morphological traits, have made it possible to evaluate genetic diversity contained within and between cultivars and have helped in identifying duplicate accessions in genebanks (Virk *et al.* 1996; Zhu 1996). However, there is comparatively little information available on intra-accession (cultivar) variation in landraces compared with variation between them. Olufowote *et al.* (1997) evaluated the within-cultivar variation in 71 phenotypically purified 71 rice varieties using a combination of RFLP and microsatellite (SSR) markers. Microsatellite has it made possible to reveal diversity even in phenotypically homogenous populations, which were not detected in the field (Olufowote *et*

*al.* 1997). Luce *et al.* (2001) used microsatellite in detecting inter- and intravarietal diversity in a collection of 419 rice accessions from genebanks in European countries and compared it with a collection of 57 Asian varieties. In the present study, levels of within- and between-cultivar variations were detected and compared among the landraces, PPB varieties and pure-line varieties under the study.

### Materials and methods

A total of 108 individual plants representing 12 rice cultivars were included in the study for within-cultivar (intrapopulation) variation. These 12 cultivars represented an array of rice germplasm: landraces, PPB varieties for high altitude and PPB bulk populations and upland rice varieties. In the second part of the study, 20 rice varieties comprised of landraces, PPB varieties and bulk populations, modern cross varieties and cross parents were included for intercultural variation (Table 1). Landraces included in the present study were randomly sampled at the household level from Jumla, Kaski and Bara districts and they were the common landraces grown by many households (HHs) in a large area (Figure 1). Each sample had a traditional name given by farmers and each name was considered as one farmers' unit of diversity (FUD) and they represented diverse rice-growing environments of study sites (Table 1).

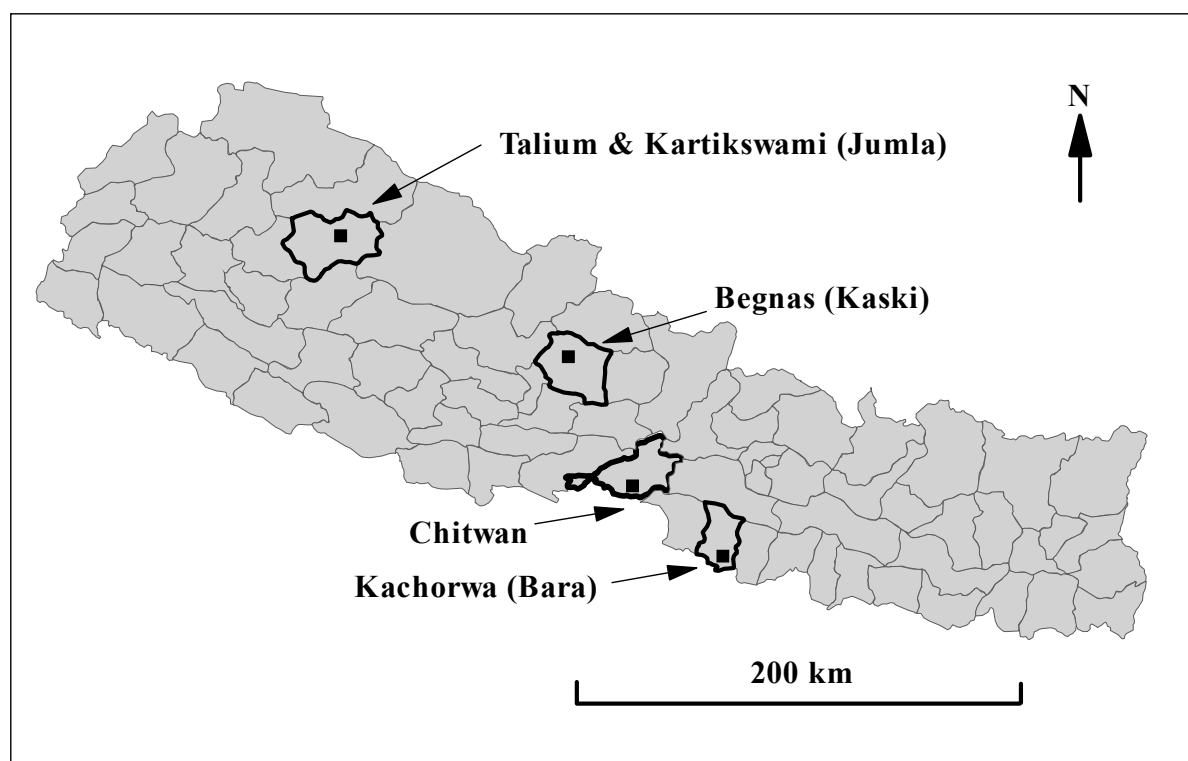
### DNA extraction

Young leaf tissue of 2–3-week-old healthy seedlings was used for genomic DNA extraction. Fresh leaf materials sampled from the 10 seedlings per accession were weighed out to 100 mg and DNA of 10 individuals and bulk of the same 10 plants was extracted using Qiagen DNeasy protocol (Qiagen 1999). The extracts were checked for DNA concentration on 0.8% agarose mini-gel in 1xTBE buffer (0.09M Tris-borate and 0.5M EDTA) at 80 volts for 1.5 hr with ethidium bromide staining.

**Table 1. Rice varieties and landraces of different origin assessed for intra- and intervarietal diversity by the use of microsatellite (SSR) markers.**

Variety	Lines	Origin	DNA sample		Remarks
Seto Marshi (1022)†	Landrace, Jumla	Nepal	NA	Bulk of 10	Cold tolerant, widely grown
Kalo Marshi (1084)	Landrace, Jumla	Nepal	NA	Bulk of 10	Cold tolerant, widely grown
Jetho Budho (207)	Landrace, Kaski	Nepal	9 individuals	Bulk of 10	Aromatic popular rice
Kathe Gurdi (029)	Landrace, Kaski	Nepal	10 individuals	Bulk of 10	Common and widely grown
Rato Anadi (094)	Landrace, Kaski	Nepal	9 individuals	Bulk of 10	Bold and glutinous rice
Basmati (320)	Landrace, Bara	Nepal	6 individuals	Bulk of 10	Aromatic rice
Nakhisar (308)	Landrace, Bara	Nepal	8 individuals	Bulk of 10	Early rice
Laltenger (104)	Landrace, Bara	Nepal	10 individuals	Bulk of 10	Late maturing rice
Machhapuchhre-3	PPB (F4 bulk)	Nepal	10 individuals	Bulk of 10	Chhomrong x Fuji 102
Machhapuchhre-9	PPB (F4 bulk)	Nepal	10 individuals	Bulk of 10	Chhomrong x Fuji 102
Chhomrong	Pure line	Nepal	10 individuals	Bulk of 10	High altitude variety
ET	PPB bulk	Nepal	8 individuals	Bulk of 10	Kalinga III x IR64 early tall
MD	PPB bulk	Nepal	NA	Bulk of 10	Kalinga III x IR64
MT1	PPB bulk	Nepal	NA	Bulk of 10	Kalinga III x IR64
MT2	PPB bulk	Nepal	NA	Bulk of 10	Kalinga III x IR64
MT3	PPB bulk	Nepal	NA	Bulk of 10	Kalinga III x IR64
Ashoka #200F	PPB (F4 bulk)	India	9 individuals	Bulk of 10	Kalinga III x IR64
Kalinga III	Pure line	India	9 individuals	Bulk of 10	Upland variety
IR64	Cross variety	IRRI	NA	Bulk of 10	Irrigated
IR36	Cross variety	IRRI	NA	Bulk of 10	Irrigated

† = Number in parenthesis indicates the accession number of landraces adopted during survey and sample collection activity carried under *In situ* conservation project, 1998-99.



**Figure 1. Map of Nepal showing the collection sites of rice landraces and PPB bulk populations.**

### Microsatellite markers

Thirty-six rice SSR primers synthesized by Research Genetics, Cornell, USA, representing different regions of the rice genome were used for the amplification of rice landraces. These microsatellite (SSR) markers were selected based on the level of polymorphism detected in landrace populations of Nepal (Bajracharya 2003). The selected markers were distributed across the genome of rice with (GA)<sub>n</sub>, (CT)<sub>n</sub>, (AT)<sub>n</sub> and (AAAT) repeats.

### PCR amplification, product separation and detection of microsatellite

The PCR reaction was conducted in a volume of 25 µl, which contained 5 ng of genomic DNA and used Ready Mix™ PCR Master mix composed of 3.0 mM MgCl<sub>2</sub>, 10 × PCR buffer, *Taq* polymerase and blue dye. PCR reaction mixtures were amplified in a MJ Research PTC – 100™ Programmable Thermal Controller with Hot Bonnet (MJ Research, INC, Waltham, MA, USA) holding 96 × 0.2 ml well V bottom PCR plate and followed the temperature cycling conditions of touchdown PCR with some modification in annealing (Cho *et al.* 2000). PCR products were separated by horizontal agarose gel electrophoresis; 12.5 µl of each PCR product was subjected to 3% high resolution Amresco SFR agarose (Anachem LTD, Luton, Bedfordshire, UK) gel electrophoresis in 1 × TAE (0.11% v/v) glacial acetic acid, 0.5M EDTA and 0.04M Tris base) buffer (Sambrook *et al.* 1989) for 3-4 hr at 90 V. A 1kb DNA ladder (Gibco-BRL, Life Technologies, Paisley, UK) was used as size standard for molecular weight (MW) estimation of PCR products. Gels were stained with ethidium bromide, visualized under UV illumination and photographed using Polaroid gel cam camera system.

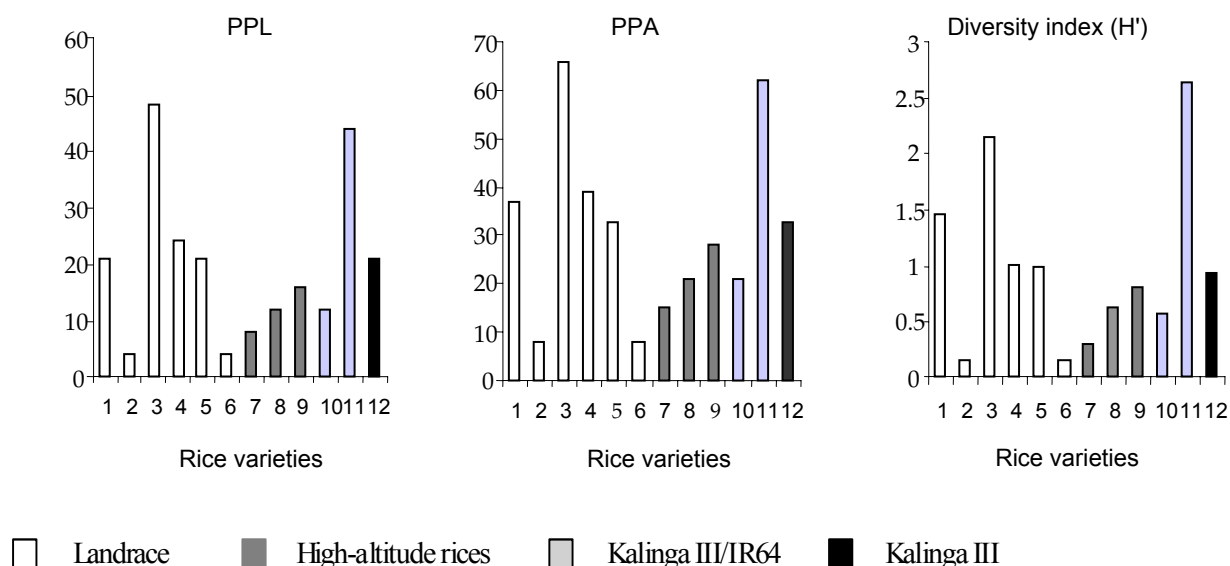
### Data analysis

The amplified fragments seen as bands on gels after staining were scored as alleles and assigned 1 for presence and 0 for absence. The approximate size of each band was determined based on its migration relative to the 1kb DNA ladder. Shannon-Weaver index values as diversity index ( $H'$ ) and other diversity parameters were calculated for each variety based on the allelic frequency produced by the markers used in the study (Shannon 1948).

### Results and discussion

#### Within-cultivar variation

Twelve rice varieties and SSR markers used in the study for within-cultivar heterogeneity at molecular level are summarized in Table 2. All the varieties with 6-10 multiple individuals per named cultivar/landrace showed within-cultivar heterogeneity at a minimum of 1 and a maximum of 12 markers out of 25 markers and revealed the maximum 3 alleles per locus. Three markers: RM229, RM234, and RM257 (12% of the total markers tested) found non-informative with no detection of within-cultivar variation in 12 varieties included in the study. *Rato Anadi* (a landrace from Kaski) and Early tall (Early Tall, a PPB bulk population of cross KIII  $\times$  IR64 selected by the farmers in Chitwan) showed highest within-cultivar heterogeneity with high diversity values (Figure 2). Two landraces—*Kathe Gurdi* and *Laltenger*—appeared most homogeneous with least (0.15) diversity index (Figure 2). The study indicated that landraces were heterogeneous and a high allelic diversity was observed as a characteristic of a landrace population. PPB bulk population (Early tall) on the other hand was also found as equally diverse as the most diverse landrace, *Rato Anadi*. However, a low heterogeneity among the individuals of PPB varieties for high-altitude (Machhapuchhre 3 and Machhapuchhre 9) was detected with obvious monomorphic alleles for most markers analyzed. A similar observation with single banding pattern (no cultivar variations) was detected in SSR diversity among rice landraces of Jumla (Bajracharya *et al.* 2005). The supposedly pure line varieties Chhomorong and Kalinga III were more diverse than the PPB varieties (Figure 2).



**Figure 2. Within-cultivar allelic variation among 12 rice varieties based on the variant individuals produced by 25 microsatellite (SSR) markers. (1. Jetho Budho; 2. Kathe Gurdi; 3. Rato Anadi; 4. Nakhisaro; 5. Basmati; 6. Laltenger; 7. Machhapuchhre 3; 8. Machhapuchhre 9; 9. Chhomorong; 10. Ashoka 200 F; 11. Early tall bulk; and 12. Kalinga III). (PPL=percentage of polymorphic loci; PPA=percentage of polymorphic alleles;  $H'$ =Shannon-Weaver Diversity Index).**

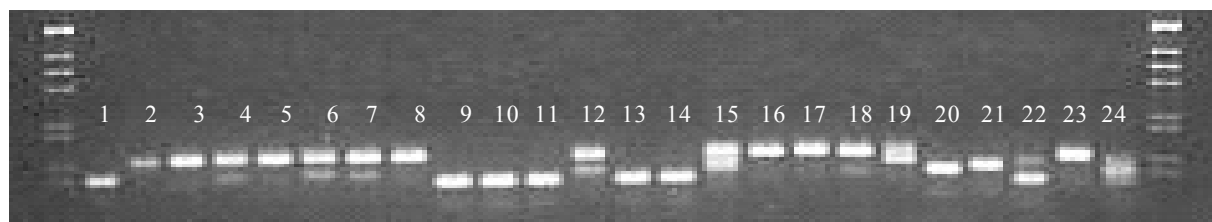
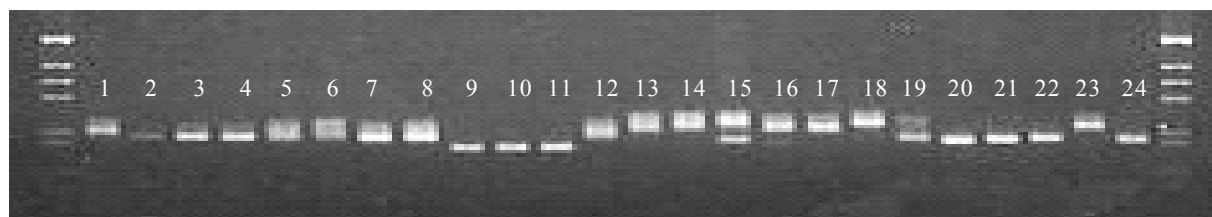
**Table 2. Proportion of alleles and Shannon-Weaver diversity indices (H') in 12 rice varieties showing within-accession variation.**

SSR locus	No. alleles	Kaski landraces			Bara landraces			PPB varieties and parental variety			PPB and parental varieties		
		JB	KG	RA	NS	BAS	LAL	M3	M9	CHH	A200F	ET	KIII
RM246	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.00	0.62	1.0
	2	0	0	0	0	0	0	0	0	0	0	0.38	0
RM5	1	0.66	1.0	0.88	0.87	0.83	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0.24	0	0.12	0.13	0.17	0	0	0	0	0	0	0
RM48	1	0.66	1.00	0.88	0.87	1.00	1.0	0.90	0.70	1.0	1.0	1.0	1.0
	2	0.24	0	0.12	0.13	0	0	0.10	0.30	0	0	0	0
RM213	1	1.0	1.0	1.0	1.0	1.0	1.0	0.90	0.80	0.80	0.88	0.62	1.0
	2	0	0	0	0	0	0	0.10	0.20	0.20	0.12	0.38	0
RM232	1	1.0	1.0	0.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.87	0.88
	2	0	0	0.12	0	0	0	0	0	0	0	0.13	0.12
RM226	1	0.66	0.90	1.0	1.0	1.0	1.0	1.0	0.90	0	0.76	1.0	0.66
	2	0.22	0.10	0	0	0	0	0	0.10	1.00	0.24	0	0.24
	3	0.12	0	0	0	0	0	0	0	0	0	0	0
RM164	1	1.0	1.0	1.0	1.0	1.0	0.90	1.0	1.0	1.0	1.0	0.63	1.0
	2	0	0	0	0	0	0.10	0	0	0	0	0.37	0
RM3	1	1.0	1.0	0.88	0.87	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0	0	0.12	0.13	0	0	0	0	0	0	0	0
RM234	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
RM248	1	1.0	1.0	1.0	0.87	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.88
	2	0	0	0	0.13	0	0	0	0	0	0	0	0.12
RM11	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.75	1.0
	2	0	0	0	0	0	0	0	0	0	0	0.25	0
RM223	1	0.66	1.0	1.0	0.87	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0.24	0	0	0.13	0	0	0	0	0	0	0	0
RM257	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
RM242	1	1.0	1.0	0.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0	0	0.12	0	0	0	0	0	0	0	0	0
RM228	1	1.0	1.0	0.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.75	1.0
	2	0	0	0.12	0	0	0	0	0	0	0	0.25	0
RM222	1	1.0	1.0	0.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0	0	0.12	0	0	0	0	0	0	0	0	0
RM206	1	1.0	1.0	0.88	1.0	0.83	1.0	1.0	1.0	0.90	1.0	1.0	1.0
	2	0	0	0.12	0	0.17	0	0	0	0.10	0	0	0
RM21	1	1.0	1.0	0.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.74	0.88
	2	0	0	0.12	0	0	0	0	0	0	0	0.13	0.12
	3	0	0	0	0	0	0	0	0	0	0	0.13	0
RM17	1	1.00	1.00	0.55	0.87	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00
	2	0	0	0.33	0.13	0	0	0	0	0	0	0.13	0
	3	0	0	0.12	0	0	0	0	0	0	0	0	0
RM224	1	0.66	1.0	1.0	1.0	0.83	1.0	1.0	1.0	1.0	0.88	0.50	1.0
	2	0.24	0	0	0	0.17	0	0	0	0	0.12	0.50	0
RM167	1	1.0	1.0	1.0	1.0	0.83	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	0	0	0	0	0.17	0	0	0	0	0	0	0
RM229	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
RM202	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.80	1.0	0.87	0.88
	2	0	0	0	0	0	0	0	0	0.20	0	0.13	0.12
RM247	1	1.0	1.0	0.88	1.0	0.83	1.0	1.0	1.0	0.80	1.0	1.0	1.0
	2	0	0	0.12	0	0.17	0	0	0	0.20	0	0	0
RM20	1	1.0	1.0	0.66	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.87	1.0
	2	0	0	0.34	0	0	0	0	0	0	0	0.13	0
H'		1.45	0.15	2.16	1.01	0.99	0.15	0.29	0.62	0.80	0.57	2.64	0.92

JB = Jetho Budho; KG = Kathe Gurdi; RA = Rato Anadi; NS. = Nakhisaro; BAS = Basmati; LAL = Laltenger; M3 = Machhapuchhre 3; M9 = Machhapuchhre 9; CHH = Chhommrong; A200F = Ashoka 200 F; ET = Early tall; and KIII = Kalinga III; † = Chromosome number.

**Between-cultivar variation**

The genetic diversity of 36 microsatellite loci was examined in a collection of 20 rice populations that represented 5 cultivar groups: IRRI breeds as checks, landraces, high-altitude PPB varieties, PPB bulks and parental pure-line varieties for intercultural variations. Of these, two markers were monomorphic with single banding pattern and 34 markers detected 109 alleles among these populations. The alleles per locus varied significantly among 20 rice varieties ( $F = 5.67$ ;  $P < 0.01$ ; Figure 3). The diversity values for these cultivar groups under study varied considerably and landraces from the Kaski and Bara ecosites and the PPB bulk populations were the most diverse (Table 3). The check varieties, consisting of two irrigated and one upland variety, were not unexpectedly diverse. Jumla landraces were the least diverse and were homogeneous for all of the markers tested. The next least diverse group was also from high altitudes (Chhommrong and two high-altitude PPB varieties).

**RM 224****RM 226**

**Figure 3. Agarose gel electrophoresis of SSR products showing the level of genetic variation diverse set of rice varieties comprising landraces, high-altitude PPB varieties, PPB bulks, pure-line varieties and cross varieties. (1. KIII; 2. IR64; 3. IR36; 4. ET; 5. MD; 6. MT1; 7. MT2; 8. MT3; 9. Machhapuchhre 3; 20 Machhapuchhre 9; 11. Chhommrong; 12. Askoka200F; 13. Seto marshi (1066); 14. Kalo marshi (1084); 15. Jhinuwa (012); 16. Kathegardi (029); 17. Rato anadi (094); 18. Jethobudho (207); 19. Aanga (219); 20. Laltenger (04); 21. Nakhisaro (08); 22. Sathi (018); 23. Basmati (020); 24. Lajhi (101).)**

The principal component analysis on 20 rice varieties showed similar groupings and relationships among the varieties as with the individuals of 12 varieties. The first two axes explained 64% of total variation (Figure 3). The Nepalese landraces and high-altitude PPB varieties (Machhapuchhre 3, Machhapuchhre 9 and Chhommrong) were clearly separated from the IRRI varieties, and the Indian PPB variety (Ashoka 200F) and PPB bulks of Kalinga III  $\times$  IR64 with exceptions of two landrace accessions (Rato Anadi from Kaski and Laltenger from Bara). It showed the clustering of the materials under study in accordance with their diverse origins.

As expected, the landraces from Bara and Kaski displayed a higher intercultural diversity than high-altitude materials. Rato Anadi (RA), an upland, late-maturing variety with glutinous rice from Kaski, and Laltenger (LAL), an irrigated, late-maturing variety from Bara, however, were observed close to the IRRI check varieties. The scatter of the varieties shows that there is intercultural variation among these varieties (Figure 4).

**Table 3. Comparative allelic diversity among different cultivar groups of rice consisting of 20 different accessions.**

Diversity parameter	Check vars. (2)	PPB bulk pop <sup>n</sup> (5)	PPB vars. (3)	Landraces			Total accessions (20)
				Jumla (2)	Kaski (3)	Bara (3)	
Rice varieties	IR36, IR64, KIII	ET, MD, MT1, MT2, MT, A200F	M3, M9, CHH	SM, KM	JB, KG, RA	NS, BAS, LAL	20 varieties
No. of markers	34	34	34	34	34	34	34
Total alleles	52	57	36	34	57	60	109
Alleles per locus	1.5	1.7	1.1	1.0	1.7	1.8	3.2
Polymorphic markers	17	19	2	0	23	22	34
Alleles per polymorphic marker	2.1	2.2	2.0	0	2.0	2.2	3.2
PPA†	67	74	11	0	81	80	100
PPL‡	50	56	6	0	68	65	100

† PPA = Percentage of polymorphic alleles.

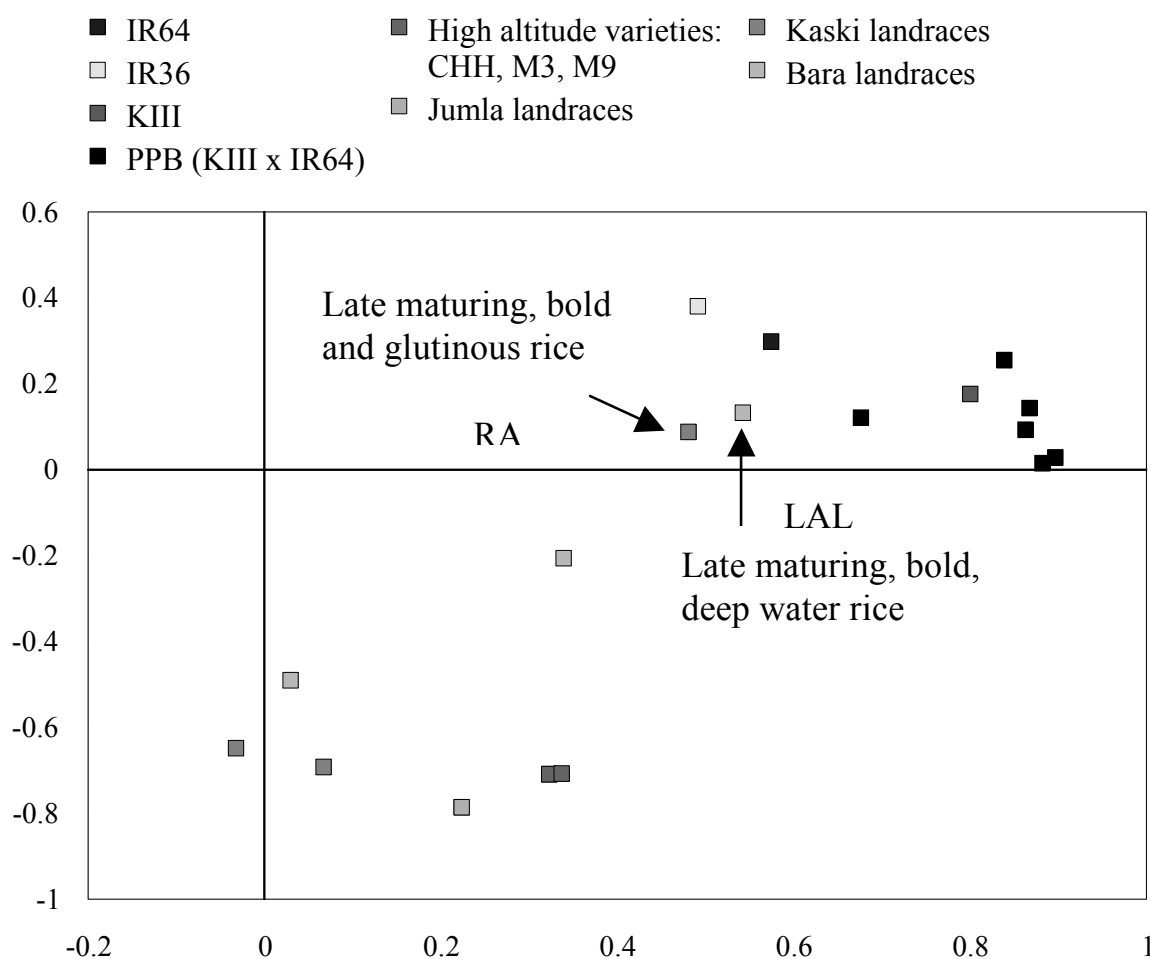
‡ PPL = Percentage of polymorphic loci.

Various molecular markers have been used to examine diversity in rice at the inter- and intravarietal and species levels (McCouch *et al.* 1988; Wang and Tanksley 1989; Yang *et al.* 1994; Virk *et al.* 1995; Zhu 1996; Qian *et al.* 2001). SSR marker technique is the one which was found informative in determining the level of polymorphism for both inter- and intravarietal heterogeneity among the rice populations of different cultivar groups. SSRs provided information on the structure of a landrace population and supported the genetic relationships and differences among cultivars of different groups. The landraces were the most heterogeneous group in the study and had high levels of polymorphism with the highest average allelic variation for both within- and between-cultivar diversity. However, the landraces from Jumla alone display little diversity. Yang *et al.* (1994) and Olufowote *et al.* (1997) indicated that landraces had a higher number of alleles than modern cultivars. However, this was observed in a collection of landraces and modern cultivars that represented a wide array of rice germplasm of the world including South-East Asia. The lower number of alleles per locus detected in the present study was in a smaller set of rice landraces from only three ecozones of Nepal. Diversity depends upon the number, choice and discriminating ability of markers, the sample size, and on how diverse is the origin of the samples.

The PPB bulk ET (early tall) from the Kalinga III / IR64 cross was as diverse as the most diverse landrace variety Rato Anadi (Figure 2 and Table 3). The breeding history of ET was such that relatively high within-cultivar diversity is not unexpected. When selection pressure in the same cross (Kalinga III × IR64) was more intense, as was the case for Ashoka 200F, even though bulk population breeding was still the selection method, within-cultivar diversity declined dramatically (Figure 1) and it was less than that of the four of the six landraces and one of its parents, Kalinga III. When PPB was applied in another situation (high-altitude rice in Nepal) the PPB varieties Machhapuchhre 3 and 9 (M3 and M9) were less variable than Chhommrong. However, the PPB had generated more diversity for this high-altitude site than was found among the landraces at high-altitude Jumla.

However, the genetic variation between bulks produced from a single cross using modified bulk population breeding (Witcombe *et al.* 2001) was almost as high, when assessed by SSR markers, as the variability found among the landraces from Kaski and Bara. Moreover, the Kaski and Bara landraces were adapted to a much greater range of physical and socioeconomic environments than the six PPB bulks, five of which were adapted to the same upland environment.





**Figure 4. Scatter plot of 20 rice varieties based on 109 alleles generated by 34 microsatellite markers.**

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## Molecular marker diversity in rice (*Oryza sativa* L.) landraces of Nepal

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### Abstract

Jumla (2200-3000 m), Kaski (700-1206 m) and Bara (100-150 m) were three study sites that represent three physiographic agroecozones of the country: *terai* (plain), mid-hill and high hill. A range of diversity in the form of rice landraces is under cultivation in traditional rice production and management systems of Nepal. These landraces are grown for diverse uses such as for home consumption, sale and for religious and cultural purposes and are adapted to a range of agroecological niches. To understand and assess the value and extent of genetic diversity prevalent in landraces, a diversity study was made on a collection of landrace accessions from three agroecozones using microsatellite (SSR) markers. Seventy rice accessions (21 from Jumla, 24 from Kaski and 25 from Bara) comprising accessions with 10 different names from each site were assayed for genetic diversity at 39 SSR marker loci using agarose gel electrophoresis. The patterns of genetic diversity revealed by SSR polymorphisms varied between the study sites and among the varieties. Landraces from Kaski and Bara showed a high genetic variation with 0.34 average Nei's gene diversity (PIC) and 0.45 genetic dissimilarity coefficient and 88% of the markers were polymorphic. Landraces in Jumla varied very little for the SSR markers studied. Only a single marker (3%) was polymorphic and that diversity was displayed by only one accession. The clustering pattern showed relationships similar to the traditional classification of landraces in Kaski and Bara identifying the groups of genotypes with same names. The most abundantly grown landraces: *Kathegurdi* and *Laltenger* had little within-cultivar variation. Landraces grown in a small area and genotypes for culinary importance (*Basmati*, *Jethobudho*, *Rato Anadi*, *Jhinuwa*, *Nakhisaro* and *Sathi*) had a comparatively large within- and between-cultivar variation. The study showed that the mid-hill ecosite with diverse agroecological environments and the lowland ecosite with the most favourable rice growing environments both conserved a high level of rice landrace diversity.

**Key words:** landraces, polymorphism, agroecozone, simple sequence repeats

### Introduction

Rice (*Oryza sativa* L.) is the main cereal grain crop of Nepal occupying over 50% of the total agricultural land (Central Bureau of Statistics 2002) and is grown from low to high altitudes with recorded cultivation at the highest elevation of 2621m (Shahi and Hew 1979). Of this area, 21% is covered by local rice. Rice holds a significant role in Nepalese agriculture with high priority (Agricultural Perspective Plan 1995) and is important in terms of area under cultivation, employment generation, and its contribution to food security at both national and household levels. The cultivation and diversity of rice in Nepal is unique. A wide range of rice diversity on-farm is under cultivation as landraces and they have long been adapted to local environments and cultural regimes, being better suited to diverse farming systems, agroecological niches, diverse sociocultural settings and the needs of farmers across all of its altitudinal range (Paudel *et al.* 1998). These landraces constitute a conspicuous source of variation for crop improvement (Zeven 1998). They are passed from generation to generation of farmers and are exposed to natural and human selections in a local environment (Teshome *et al.* 1997). These landraces are the only resource available in a resource-poor environment and this genetic variation could be exploited in rice breeding where access to new technology is difficult (Witcombe 1999).

Agromorphological characteristics have long been used to classify and distinguish plant genotypes. In recent years, development of DNA marker technology has provided an

efficient tool to evaluate and measure the genetic diversity and cultivar identity (Ni *et al.* 2002). Of the molecular markers, microsatellite markers are one of the most powerful new genetic markers. Hundreds of microsatellites from rice have been developed and mapped (Wu and Tanksley 1993; Paunad *et al.* 1996; Chen *et al.* 1997; Temnykh *et al.* 2000).

They are based on the number of simple sequence DNA repeats (SSRs) and have become the markers of choice for a wide spectrum of genetic and population studies (Gao *et al.* 2002). Compared with morphological analysis, molecular markers can reveal differences among accessions at DNA level and provide a more reliable and efficient tool for germplasm evaluation. Microsatellites (SSRs) have been used extensively in molecular characterization and identification of rice accessions (Virk *et al.* 1995; Olufowote *et al.* 1997); determination of genetic structure and pattern of diversity (Zhang *et al.* 1992; Akagi *et al.* 1997) and conservation and management of genetic resources (Akagi *et al.* 1996; Ford-Lloyd *et al.* 1997). Nepalese landraces with different names tended to be morphologically distinct in agromorphological observation (Bajracharya 2003). However, very little is known about the genetic structure and relationships of these rice landraces. The purpose of this study was therefore to measure and evaluate genetic diversity in landrace accessions of rice from three major agroecosystems of Nepal using SSR markers.

### Materials and methods

Jumla (2240-3000 masl), Kaski (668-1206m asl) and Bara (100-150 masl) are three study sites selected to represent three major physiographic agroecozones of the country (Figure 1). A collection of diverse rice accessions from each site was collected at household level using random strata of wealth. Based on area under cultivation (large/small) and number of households (many/few) growing a named landrace, 21 rice accessions from high hills (Jumla), 24 from mid-hills (Kaski) and 25 from lowland (Bara) were included in SSR analysis. These were randomly sampled from the total collections of each site. The sampled accessions of each site were comprised of 10 differently named landraces (Table 1). Three modern varieties (Kalinga III, IR64 and IR36) were included as reference cultivars for SSR analysis.

### DNA extraction

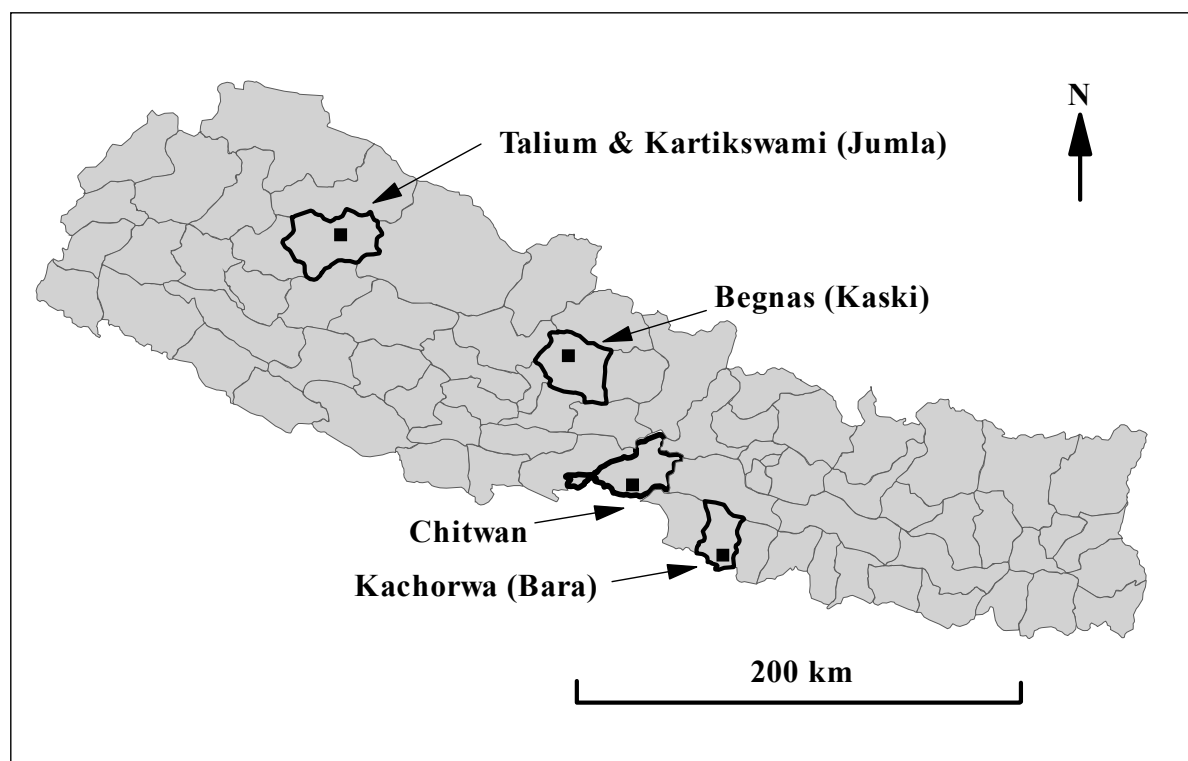
Young leaf tissue of 2–3-week-old healthy seedlings was used for genomic DNA extraction. Bulk DNA of 10 individual seedlings per accession was extracted using Qiagen DNeasy protocol (Qiagen 1999) and checked for DNA concentration on 0.8% agarose mini-gel in 1xTBE buffer (0.09M Tris-borate and 0.5M EDTA at 80 volts for 1.5 hr with ethidium bromide staining).

### Microsatellite markers

Forty rice microsatellite (SSR) primers synthesized by Research Genetics, USA, representing different regions of the rice genome were used for the amplification of rice landraces. These microsatellite (SSR) primers were selected based on the level of published polymorphic information content. The selected markers were of (GA)<sub>n</sub>, (CT)<sub>n</sub>, (AT)<sub>n</sub> and (AAAT) repeats.

### PCR amplification

For SSR analysis, the protocol developed by McCouch *et al.* (1997) was followed with minor modifications. The PCR reaction was conducted in a volume of 25 µl, using Ready Mix™ PCR Master mix composed of 3.0 mM MgCl<sub>2</sub>, 10 x PCR buffer, *Taq* polymerase and blue dye amplified in a MJ Research PTC-100™ Programmable Thermal Controller with Hot Bonnet (MJ Research, INC, Waltham, MA, USA). The temperature cycling conditions programmed for amplification followed Cho *et al.* (2000). It was 5 min at 94°C followed by 2 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 2 min; then 2 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 2 min; then 4 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 2 min; followed by 25 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 2 min and finally 72°C for 5 min then 4°C hold.



**Figure 1. Map of Nepal showing the study sites in three agroecozones.**

#### **PCR product separation and detection of microsatellite**

For each PCR product, 12.5 µl was subjected to 3% high resolution Amresco SFR agarose (Anachem LTD, Luton, Bedfordshire, UK) gel electrophoresis in 1 x TAE (0.11% v/v) glacial acetic acid, 0.5M EDTA and 0.04M Tris base) buffer (Sambrook *et al.* 1989). A 1kb DNA ladder (Gibco-BRL, Life Technologies, Paisley, UK) was used as size standard. After electrophoresis, gels were stained with ethidium bromide and photographed using Polaroid gel cam camera system.

#### **Data analysis**

The amplified fragments seen as bands on gels after staining were scored as alleles and assigned 1 for presence and 0 for absence. The approximate size (in nucleotides as base pairs) of each band was determined based on its migration relative to the 1kb DNA ladder. Polymorphic information content (PIC) values also called Nei's gene diversity were calculated for each marker based on the allelic frequency (Nei 1973). The data were statistically analyzed to detect relationships among accessions and landrace cultivars within and between the sites using multivariate analysis. All the polymorphic SSR alleles were subjected to cluster analysis (except for the Jumla site where both polymorphic and monomorphic alleles were used) using the NTSYS-pc program (Rohlf 1990). Dendograms were created based on Nei's genetic distance using an Unweighted Pair Group Method with Arithmetical Averages (UPGMA). Principal component analysis (PCA) was done on the pooled data to show the genetic relationships among the ecogeographic regions.

Table 1. Rice genotypes used in the study on DNA variation by SSR markers

Jumla ecosite			Kaski ecosite			Bara ecosite		
Landrace	Accession no.	Remarks Area/HH <sup>†</sup>	Landrace	Accession no.	Remarks Area/HH <sup>†</sup>	Landrace	Accession no.	Remarks Area/HH <sup>†</sup>
Kalinga III	Check variety	MV	Kalinga III	Check variety	MV	Kalinga III	Check variety	MV
IR64	Check variety	MV	IR64	Check variety	MV	IR64	Check variety	MV
IR36	Check variety	MV	IR36	Check variety	MV	IR36	Check variety	MV
Darime	1052	Small/few	Kathe Gurdi	25KG	Large/many	Mutmur	65M	Large/many
Darime	2006	Small/few	Kathe Gurdi	28KG	Large/many	Mutmur	66M	Large/many
Jumli R. Marshi	2036	Small/few	Kathe Gurdi	29KG	Large/many	Mutmur	301M	Large/many
Kalo Marshi	1023	Large/many	Kathe Gurdi	33KG	Large/many	Nakhisar	118N	Large/many
Kalo Marshi	1057	Large/many	Jetho Budho	203JB	Large/many	Nakhisar	117N	Large/many
Kalo Marshi	1084	Large/many	Jetho Budho	205JB	Large/many	Nakhisar	308N	Large/many
Mehele	2051	Small/few	Jetho Budho	207JB	Large/many	Sathi	12S	Small/many
Mehele	2068	Small/few	Jetho Budho	214JB	Large/many	Sathi	18S	Small/many
Palte Dhan	2020	Small/few	Gurdi	58G	Large/few	Sokan	183So	Small/few
Rato Dhan	1020	Small/few	Rato Anadi	92RA	Small/many	Sokan	307So	Small/few
Rato Marshi	1009	Large/many	Rato Anadi	94RA	Small/many	Mansara	146Ma	Large/many
Rato Marshi	1015	Large/many	Rato Anadi	104Ra	Small/many	Mansara	150Ma	Large/many
Rato Marshi	2003	Large/many	Aanga	219A	Small/many	Mansara	148Ma	Large/many
Rato Marshi	2035	Large/many	Aanga	224A	Small/few	Basmati	52B	Small/many
Seto Dhan	2033	Small/few	Ramani	158R	Small/few	Basmati	44B	Small/many
Dhan	1001	Small/few	Ramani	161R	Small/few	Basmati	320B	Small/many
Dhan	1013	Small/few	Seto Gurdi	52SG	Small/few	Karma	36K	Small/many
Seto Marshi	1022	Large/many	Seto Gurdi	53SG	Small/few	Karma	33K	Small/many
Seto Marshi	1064	Large/many	Jhinuwa	12J	Small/few	Karma	29k	Small/many
Seto Marshi	1066	Large/many	Jhinuwa	13J	Small/few	Lajhi	101L	Large/few
Seto Marshi	1024	Large/many	Jhinuwa	14TJ	Small/few	Lajhi	104L	Large/few
			Tunde Jhinuwa	15TJ	Small/few	Lajhi	97L	Large/few
			Kalo Bayarni	70KB	Small/few	Dudhraj	215D	Small/few
			Kalo Bayarni	72KB	Small/few	Laltenger	304La	Small/few
						Laltenger	125La	Small/few

<sup>†</sup> = area coverage large/small and number of households (HH) many/few compared with average values.

## Results

A preliminary PCR amplification was carried out using template bulk DNA of 10 individuals of each of two medium tall lines of a cross Kalinga III × IR64 (# MT2-3 and # 128-3) from a participatory plant breeding programme in Nepal (Witcombe *et al.* 2001) along with check modern varieties (MVs): Kalinga III, IR64 and IR36 and protocol was determined for successive amplification of PCR products of landraces.

### SSR diversity and genetic relationships in the Jumla landraces

Out of 40 markers tested, 39 markers yielded the amplification products in all accessions under study, except accession 1052 and it was excluded from the diversity analysis. A total of 81 alleles (bands) at 39 loci were detected with an overall average of 2.1 alleles per locus in the whole set (20 landraces and 3 MVs) of rice accessions and 40 alleles in 20 landrace accessions (Table 2). Thirty-two primer pairs (82%) detected DNA polymorphism among 23 rice accessions (PIC 0.08). However RM226 (3%) was the only polymorphic locus with two alleles in an accession (1013) with 0.05 PIC (Figure 2a). Polymorphisms between the modern check varieties and landrace accessions were observed at each of 32 loci out of the 39 primer pairs. Landraces from Jumla, although they were called by different traditional names, were found to be genetically homogenous with a common allele for all 39 SSRs markers tested. The observed 81 alleles (polymorphic and monomorphic) were subjected to multivariate analyses. All the landrace accessions clustered together and formed a distinct group from the check modern varieties (Figure 3). The landraces from Jumla, all but those with different traditional names, were genetically homogenous with the sole exception of 1013.

**Table 2. Number of alleles and polymorphic information content (PIC) in rice accessions from the Jumla site.**

Diversity parameters	Total sample	Landrace accessions	Check varieties
Total number of alleles	81	40	57
Total number of accessions	24	20	3
Average alleles per locus	2.1	1.0	1.5
Number of polymorphic loci	32	1	17
Number of polymorphic alleles	74	1	35
Average alleles per polymorphic locus	2.3	2.0	2.1
PPL <sup>†</sup>	82.1	2.6	43.6
PPA <sup>‡</sup>	91.3	5.0	61.5
Average gene diversity (PIC)	0.17	0	

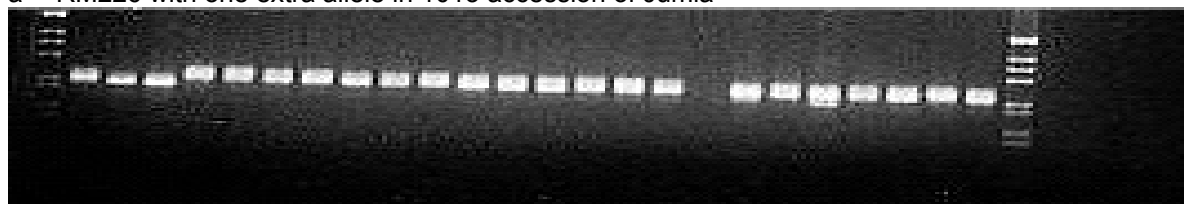
<sup>†</sup> PPL = percentage of polymorphic loci.

<sup>‡</sup> PPA = percentage of polymorphic alleles.

### SSR diversity and genetic relationships in the Kaski landraces

Among 39 SSR markers tested, RM201, with no amplified product in most accessions of Kaski was excluded from this diversity analysis. With the remaining 38 primer pairs 105 alleles were scored in the whole set of 27 rice landraces and checks and 91 alleles in the 24 landraces (Table 3). Out of 38 primer pairs, 95% detected polymorphisms in the whole set of 27 accessions including checks and 90% in 24 landrace accessions. The number of alleles observed at each locus ranged from 1 (RM60 and RM120) to 6 (RM247) with an average of 2.7 alleles per locus for the whole set, 2.4 for the landrace set and 1.5 for check modern varieties. The maximum number of alleles for landraces was 5 alleles with RM247 (Figure 2b). Four alleles were resolved with markers RM246, RM164 and RM20 while the rest of the markers resolved 2-3 alleles, and some were monomorphic. The average PIC values as gene diversity estimated over 27 accessions and 24 accessions (without check varieties) were 0.39 and 0.33 respectively (Table 3).

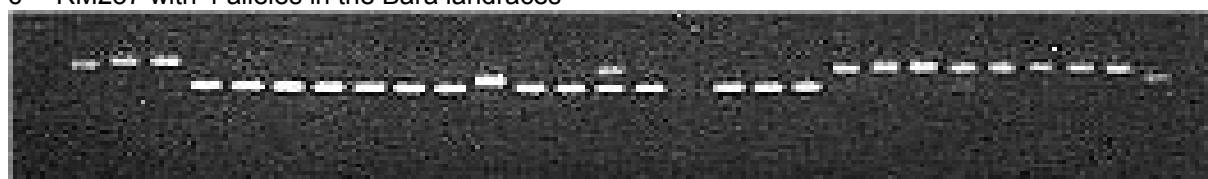
a RM226 with one extra allele in 1013 accession of Jumla



b RM247 with 5alleles in the Kaski landraces



c RM257 with 4 alleles in the Bara landraces



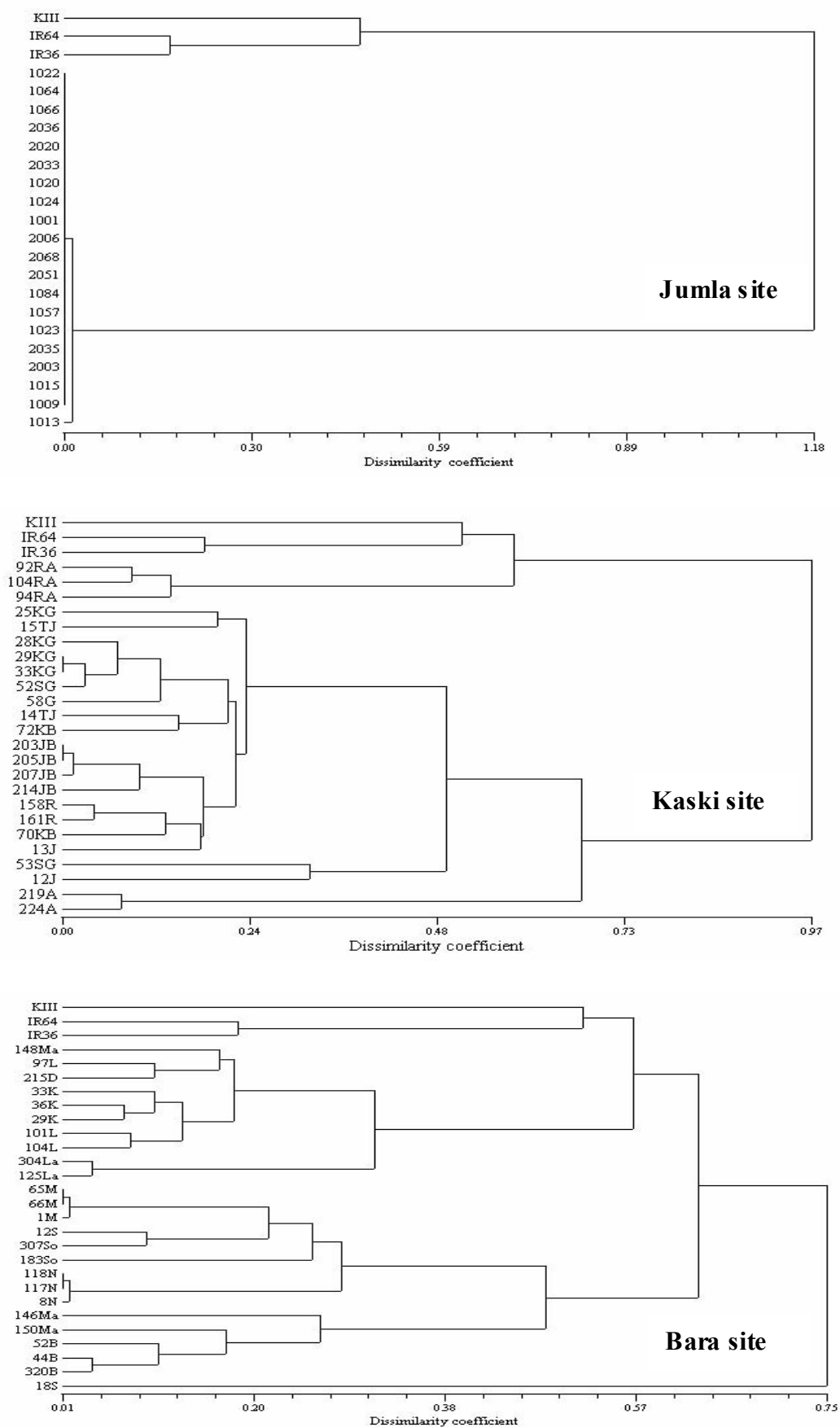
**Figure 2. Microsatellite DNA polymorphism in rice landraces showing banding patterns revealed.**

The clustering of rice landraces from Kaski site based on 103 polymorphic alleles showed that landraces had a considerable level of genetic diversity within and between different named landraces. With the genetic dissimilarity coefficient  $<0.24$ , the 27 rice accessions could be divided into 7 clusters by UPGMA (Figure 3) and were found consistent with ethnobotanical classifications adopted by the farmers. Landraces named *Rato Anadi*, *Jethobudho*, *Ramani*, *Anga* and *Gurdi* clustered close together and formed individual groups. However, *Jhinuwa*, *Tunde Jhinuwa*, *Seto Gurdi* and *Kalo Bayarni* did not cluster in groups and showed variation among landraces with the same name. Among 24 landrace accessions, two accessions of *Kathe Gurdi* (29KG and 33KG) and two accessions of *Jethobudho* (203JB and 205JB) were observed genetically identical. *Aanga* (219A and 224A) and *Rato Anadi* (92RA, 94RA and 104RA) formed the distinct clusters.

### SSR diversity and genetic relationships in the Bara landraces

All the Primer pairs except RM201 produced prominent PCR products in all 27 rice accessions of Bara. With the remaining 38 primer pairs, 102 alleles were detected in the whole set of 28 landraces and checks; 95 alleles in 25 landraces; and 56 in 3 check rice varieties (Table 4). Number of alleles at each of locus ranged from 1 to 4 with an average of 2.7 for whole set; 2.5 for the landraces; and only 1.5 for the 3 check varieties. A maximum of four alleles were resolved at each of 7 loci. Two to three alleles were identified for most of the loci (Figure 2c). RM122, RM22, RM60, RM120 and RM244 were monomorphic for all the rice accessions from Bara. PIC values were relatively high for most loci, averaging 0.35 for landraces alone and 0.40 for the whole set of landraces and check MVs. Rice accessions from Bara were found distributed in 5 major clusters at the dissimilarity level  $<0.28$  (Figure 3). The clustering clearly discriminated the early and normal to late rice varieties. These were in a cluster of 10 accessions of normal rice varieties (*Lajhi*, *Dudhraj*, *Karma*, *Mansara* and *Laltenger*), a cluster of five accessions of normal rice varieties (*Basmati* and *Mansara*) and a cluster of nine





**Figure 3. Individual dendrograms of landrace accessions (Jumla, Kaski and Bara sites) and 3 check MVs.**

accessions of early maturing varieties. The early varieties could be further differentiated into three subclusters: *Mutmur* accessions; *Nakhisaro* accessions; and *Sathi* (12S), *Sokan* (307So) in a separate group. An accession of *Sokan* (183) and *Sathi* (18S) were two early rice varieties which separated clearly from other accessions and did not fall in any of the clusters. Two accessions of *Mutmur* (65M and 66M) and two accessions of *Nakhisaro* (117N and 118N) were identical to each other.

**Table 3. Number of alleles and polymorphic information content (PIC) in rice accessions from the Kaski site.**

Diversity parameters	Total sample	Landrace accessions	Check varieties
Total number of alleles	105	91	56
Total number of accessions	27	24	3
Average alleles per locus	2.7	2.4	1.5
Number of polymorphic loci	36	34	16
Number of polymorphic alleles	103	87	34
Average alleles per poly. locus	2.8	2.6	2.1
PPL <sup>†</sup>	94.7	89.5	42.1
PPA <sup>‡</sup>	98.1	95.6	60.7
Average gene diversity (PIC)	0.39	0.33	

<sup>†</sup> PPL = percentage of polymorphic loci.

<sup>‡</sup> PPA = percentage of polymorphic alleles.

**Table 4. Number of alleles and polymorphic information content (PIC) in rice accessions from the Bara site.**

Diversity parameters	Total sample	Landrace accessions	Check varieties
Total number of alleles	102	95	56
Total number of accessions	28	25	3
Average alleles per locus	2.7	2.5	1.5
Number of polymorphic loci	36	33	16
Number of polymorphic alleles	100	90	34
Average alleles per poly. locus	2.8	2.7	2.1
PPL <sup>†</sup>	94.7	86.8	42.1
PPA <sup>‡</sup>	98.0	94.7	60.7
Average gene diversity (PIC)	0.40	0.35	

<sup>†</sup> PPL = percentage of polymorphic loci.

<sup>‡</sup> PPA = percentage of polymorphic alleles.

### **Comparison of genetic diversity between landraces from three ecosystems**

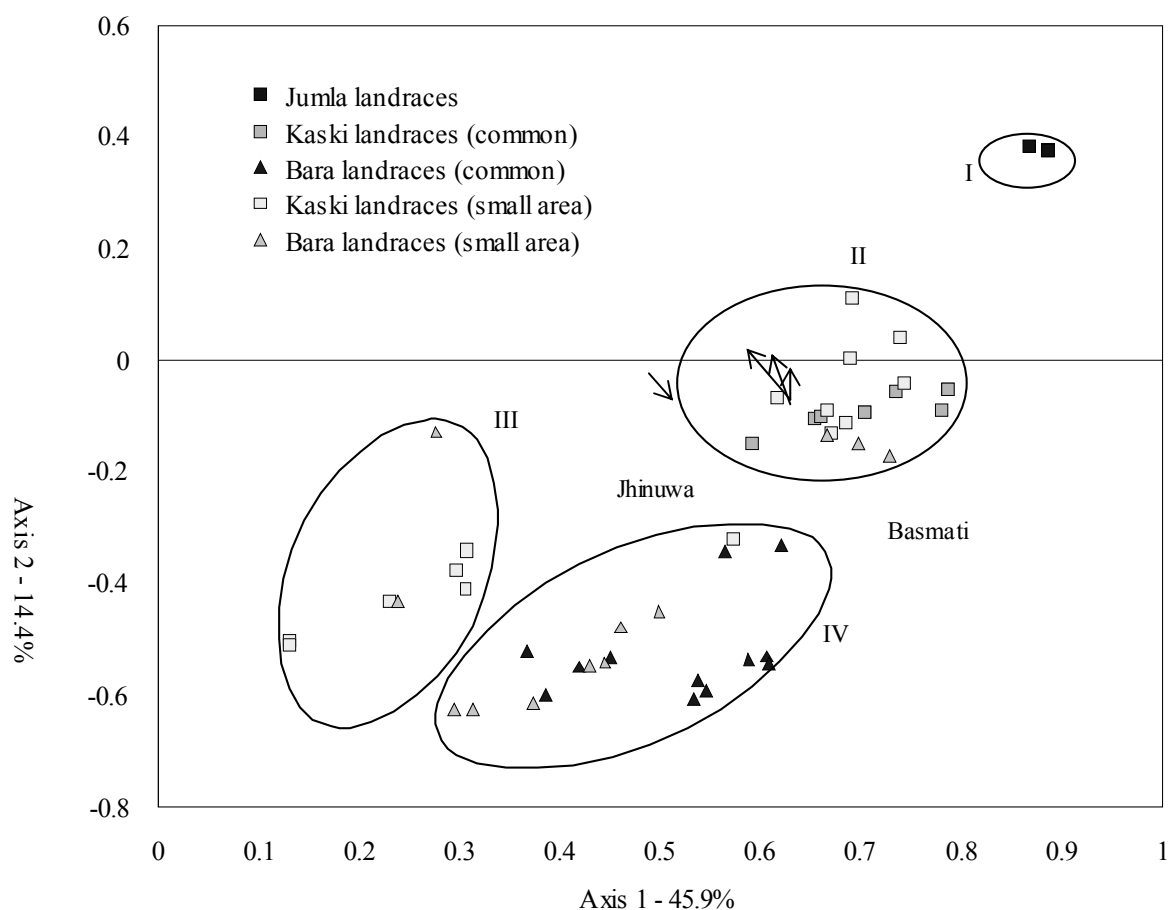
A comparison of the genetic diversity of rice accessions was performed among three sets of landraces from three different agroecosystems of the country. Landraces from the mid-hill (Kaski) and lowland (Bara) were more diverse, had a significantly greater average number of alleles per locus ( $P < 0.0001$ ) and higher PIC values ( $P < 0.0001$ ) than the landraces from the high-hill site (Jumla) (Table 5). The average gene diversity (PIC) values were found significantly correlated with the number of alleles across the sites as expected (0.78, Jumla; 0.69, Kaski; and 0.73, Bara;  $P < 0.01$ ). The average genetic dissimilarity coefficients showed that the Kaski and Bara landraces equally diverse and much more so than the Jumla landraces (Table 5).

PCA based on the SSR markers grouped all 70 landraces of three sites into 4 clusters and 60% of the total variation was explained (Figure 4). The Jumla landraces made a highly distinct single cluster and were genetically different from the landraces of Kaski and Bara. However, the Bara and Kaski landraces were less ecogeographically differentiated and were distributed in adjacent clusters. But these markers were found powerful that made distinction between the fine grained aromatic and coarse grained glutinous rice varieties. The less common rice varieties (small and few HH) were more diverse than the common ones:

the landraces from Kaski and Bara sites belonging to this category were found distributed into three adjacent clusters.

**Table 5. Summary of microsatellite polymorphism in rice landraces and their comparison between three ecosites.**

Diversity parameters	Jumla	Kaski	Bara
Total size of accessions	20	24	25
Total number of primers	39	38	38
Total number of polymorphic primers detected	1	34	33
Total number of bands amplified	40	91	95
Average number of bands per primer	1.0	2.4	2.5
Maximum number of bands amplified by a single primer	2	5	4
Minimum number of bands amplified by a single primer	1	2	2
Number of polymorphic bands identified	2	87	90
Percentage of polymorphic bands (PPA)	5.0	95.6	94.7
Average number of polymorphic bands per primer	2.0	2.6	2.7
Average genetic dissimilarity	0.12	0.45	0.45
Average gene diversity (PIC)			
(a) all loci	0.00	0.33	0.35
(b) Polymorphic loci	0.05	0.37	0.40



**Figure 4. Scatter plot of 69 rice landraces based on PCA of 117 microsatellite alleles.**

## Discussion

Each of the primer pairs detected variation at a single locus with alleles varying from 1–6 per locus and 33 markers showed polymorphism in landrace genotypes and gave an understanding of genetic relationships and diversity among these landraces from different agroecosystems of the country. The patterns of genetic diversity varied within each study site. A much larger genetic diversity as PIC (0.33–0.35 PIC) was detected in landraces from Bara and Kaski and 87–90% of markers were able to detect the genetic variation. It was found consistent with traditional classifications of landraces, each with a given name. Very little variation was encountered in landraces from Jumla. Only a single marker (3%) was polymorphic and that diversity was displayed by only one accession. Indeed, rice landraces from the high-hill (Jumla) site had a narrow genetic base and showed homogeneity in their allelic composition with common allele for all SSR markers analyzed. In contrast, PIC was recorded as high as 0.72 for the landraces from Kaski. Gene diversity (PIC) increased with the number of alleles at a locus and showed a significant correlation across the sites. However, diversity levels measured in this study are much lower than those reported from previous studies using SSRs and other markers in varied set of rice samples (Wang and Tanksley 1989; Zhang *et al.* 1992; Xiao *et al.* 1996; Zhu *et al.* 1998; Davierwala *et al.* 2000; Qian *et al.* 2001; Blair *et al.* 2002). Yang *et al.* (1994) detected up to 25 diverse alleles for a single SSR marker (RM163) in 140 rice landraces from various parts of China, Japan and India. Thanh *et al.* (1999) also showed significant variation in microsatellite DNA polymorphisms among 31 upland rice accessions of Vietnam.

The landraces were, as expected, clearly distinct from the check modern varieties. The landraces in Jumla clustered into a single group irrespective of named landraces, and were not in agreement or consistent with traditional classification. Sebastian *et al.* (1998) in an assessment of diversity and identity of farmers' rice varieties of Philippines using microsatellites and Busso *et al.* (2000) in pearl millet found a similar disagreement. Variation in landraces was not related to the names but was more related to the farmers, who managed the seed according to traditional practices of seed management.

However, the cluster analyses of the landraces from Kaski and Bara (Figure 3) supported the traditional classification of landraces. It was apparent among landraces from the Kaski site where landraces named *Rato Anadi*, *Aanga*, *Jetho Budho*, *Raman*, and *Gurdi* formed distinct clusters. Similarly, *Basmati* and *Laltenger* from Bara formed distinct subclusters. In Bara, the clustering was more pronounced by the growth duration of the landraces. However, the grouping of some accessions with the same name was not consistent in both Kaski and Bara. These accessions tended to be the landraces grown in small area by few HHs; this could be a result of intravarietal variation, or simply the misnaming of landraces by farmers. The most abundantly grown genotypes had little variation within a named group as they clustered together but the genotypes grown in small areas and by only a few households—such as *Sathi*, *Sokan* from Bara and *Jhinuwa*, *Seto Gurdi* from Kaski—exhibited more variation and they were dispersed along the axes.

In the present study, genetic diversity of rice was recorded highest in mid-hill and lowlands with favourable and diverse rice domains. It decreased from high hill to mid-hill and lowland. The low diversity in Jumla could probably be due to a harsh environment with chilling temperatures and also possibly to the founder effect of a single population of *Jumli Marshi* from which, by continuous selection for major gene characteristics, a morphological range of landraces has evolved. The present study, however, involved the assay of a limited number of markers, so it is important to assay a large number of landrace populations and markers.

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## Participatory plant breeding conserves local landraces at genetic level: a study of molecular markers to detect parental contribution in PPB progenies

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### Abstract

The participatory plant breeding (PPB) approach encourages farmers to search, select and maintain populations with preferred traits in target environment. PPB has been considered a strategy of *in situ* conservation and promotion of local genetic resources at genetic level under the global project. PPB in rice for *in situ* conservation and promotion of landraces was initiated in Kaski and Bara sites of Nepal in 1999. In Bara, *Biramphool/Himali*, *Manasara/Khumal 4*, *Thulogurdi/NR10286*, *Pusa Basmati-1/Jethobudho*, *Pusa Basmati-1/Jethobudho* EPD, *Sanogurdi/NR10285*, *Naulomadhese/IR36* and *Ekle/Khumal 4* in Kaski and *Dudhisaro/BG1442*, *Mansara/IR62161*, *Lalka Basmati/IR62161*, *Lajhi/Rampur Masuli* and *Lajhi/IR62161* were the crosses made to meet the goal of PPB set by the farmers. Successive selections in segregating populations of early generations have identified the desirable progenies like local parents with improvement in negative traits. Eight PPB progenies of F4-F6 generations selected in Kaski and five in Bara of F4 generations were therefore examined for molecular genetic diversity and its relationships with their parents at 12 SSR (Simple Sequence Repeat) loci. The progenies of *Ekle/Khumal 4* in Kaski and *Lalka Basmati/IR62161* in Bara encountered high genetic similarity (0.88 and 0.72, respectively) to their local parents. However, the progenies of *Naulomadhese/IR36* cross in Kaski showed the highest parental contribution of IR36 with similarity coefficient of 0.67. *Jethobudho/Pusa Basmati-1*, *Manasara/Khumal-4* in Kaski and *Mansara/IR62161* were encountered with new alleles different from their parents and this showed the tendency for increase in genetic diversity. This is a preliminary observation; however, to envisage and interpret the impact in detail, there is a need to study more markers covering the whole rice genome.

**Key words:** *In situ* conservation, participatory plant breeding, landraces, simple sequence repeats, parental contribution

### Introduction

Participatory Plant Breeding (PPB) is increasingly being used for decentralized crop improvement and has been successful in several countries and with different crops (Sthapit *et al.* 1996; Witcombe *et al.* 1996; Witcombe 2001). It has been considered as a technical strategy to conserve local genetic resources that are endangered or are on the verge of extinction from their habitat by adding value to them. The local genetic resources that are grown and maintained by the farming communities as landraces on-farm with diverse sociocultural and agroecological conditions constitute a conspicuous source of variation and carry the traits which are desirable and preferred for the local environments. The IPGRI global project on *in situ* conservation has been successful in demonstrating the value of these landraces and has employed different innovative participatory approaches that could add value to these landraces through the process (Joshi *et al.* 2000). PPB is the approach that is tried in *in situ* ecosites of Nepal with the rice crop to create skills for the farmers in searching for new diversity, selection and exchange of variable populations that match their local preferences and needs. PPB has therefore been advocated as a way to maintain or even enhance the level of genetic diversity on-farm, a way to add values to traditional landraces, and helps in screening and breeding divergent local cultivars for the target environment.

Rice is one of the most agronomically and nutritionally important food crops in Nepal. It is a self-pollinating crop. The plant mating system is crucial and affects the predicted outcome of changes in crop improvement systems.

There is evidence that self-pollinating crops such as barley and rice in isolated or marginal areas may have landraces closely adapted to those areas and the switch from traditional populations to PPB effectively increases gene flow. On the other hand the maize system is relatively open with high levels of seed exchange, and a switch to PPB may require more efficient mass selection and less gene flow, both of which can lead to less diversity (see Gyawali *et al.*, Volume II, p. 202). The present study is therefore preliminarily aimed at determining if PPB conserves the local rice landraces at the genetic level and also produce the seeds of segregating progenies with increase in diversity on-farm. However, there is a need to measure the impact on the change of genetic diversity over time at village and landscape levels.

## Materials and methods

### Plant materials

Thirteen PPB progenies of rice crosses made in Kaski and Bara were used in the present study (Table 1). The preferred traits and negative traits of landraces and the improved varieties used in the crosses are shown in Table 1 and the crosses were expected to improve the negative traits of landraces through incorporation of genetic constituents of improved varieties used.

**Table 1. Names of PPB crosses, their generations and description of traits of local parents used for PPB in Kaski and Bara.**

Cross	Generation	Preferred traits of local parent of the cross	Negative traits of local parent of the cross
Kaski ecosite			
Biramphool/Himali	F <sub>5</sub>	Fine and aromatic rice, good cooking and eating qualities, adapted to lowlands (Dhab) and low hills	Short panicle, grain sterility, low yield
Mansara/Khumal 4	F <sub>5</sub>	Tolerant to drought and poor soil fertility (marginal) conditions	Poor eating qualities, low yield
Thulogurdi/NR10286	F <sub>4</sub>	Higher straw yield and excellent for mat making	Low tillering, poor response to fertilizer
Pusa Basmati-1/Jethobudho EPD	F <sub>6</sub>	High cooking quality, aromatic, good straw yield, cool water tolerant	Lower yield, blast susceptibility, susceptible to lodging
Pusa Basmati-1/Jethobudho	F <sub>6</sub>	High cooking quality, aromatic, good straw yield, cool water tolerant	Lower yield, blast susceptibility, susceptible to lodging
Sanogurdi/NR10285	F <sub>4</sub>	Good postharvest qualities, soft straw	Low tillering, grain sterility
Naulomadhese/IR36	F <sub>4</sub>	Adapted to rain-fed and hill farming, easy threshability	Low yielder and poor response to fertilizers, higher sterility
Ekle/Khumal 4	F <sub>5</sub>	High tillering, high yielding, tall plant and good straw, good postharvest qualities, adapted to hills	Sparse grain in panicle, poor adaptation to warm water and long maturity, susceptible to drought
Bara ecosite			
Didhisaro/BG 1442	F <sub>4</sub>		
Mansara/IR 62161	F <sub>4</sub>		
Lalka Basmati/IR 59606	F <sub>4</sub>		
Lajhi/Rampur Masuli	F <sub>4</sub>		
Lajhi/IR 62161	F <sub>4</sub>		



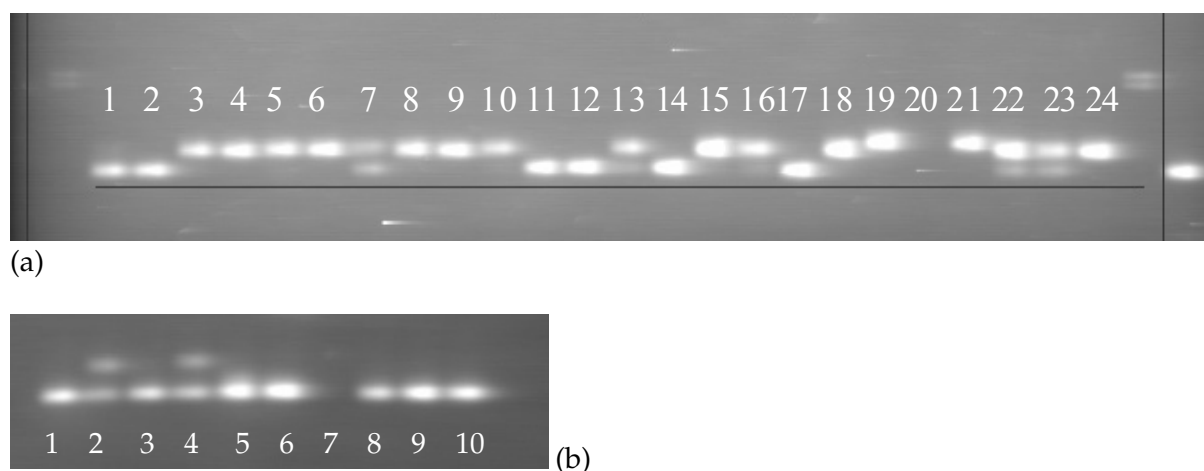
### SSR analysis

Bulk extraction of genomic DNA from young leaf tissues of 10 seedlings was carried out using Qiagen's method (1999) for each PPB cross and use of the polymerase chain reaction followed by the standard procedures used for SSR analysis. Twelve SSR primers that were earlier reported to be polymorphic and produced good amplification in rice landraces of Nepal were used for molecular analysis of these PPB progenies (Bajracharya 2003). These primers represented the rice genome. The DNA products from PCR amplification were separated on 3% agarose gels in 1 x TAE buffer and visualized by ethidium bromide staining and photographed under UV light. For each primer, the PCR products were sequentially designated as a, b, c, based on the mobility of bands. Data were scored on the presence or absence of amplification products. If the product was present in a genotype it was designated as 1 and if absent it was designated as 0. Sharing of the fragments (alleles/bands) with the respective cross parents, percentage of new alleles, and percentage of heterozygous were computed and, based on the shared fragments, the percentage of parental contribution to the progenies was calculated. Pair-wise comparisons of each cross progeny with the respective parent genotypes based on shared and new allelic data were made and similarity coefficients were generated to determine the level of genetic relationships and diversity from their cross parents (Jaccard 1908). Based on the allelic information, a diagrammatic representation of genotype of cross progenies and the parents was sketched on colour charts.

### Results and discussion

In this preliminary study, 12 SSR primers distributed in rice genomes were examined with 8 cross-products of Kaski landraces and 5 cross-products of Bara landraces along with their respective local and improved parents. Altogether 24 rice genotypes from Kaski and 10 genotypes from Bara were included in the study examining the parental contributions based on molecular information. Unfortunately, the improved male parents of the crosses of Bara landraces could not be included in the study. All the primers produced good amplification products, some shared with either parents and some found polymorphic to their parents. Figure 1 shows the primers RM234 and RM167 amplification products of 34 rice genotypes comprised of PPB progenies, landraces of Kaski and Bara (local parents) and improved varieties used in PPB process resolved by agarose gel electrophoresis.

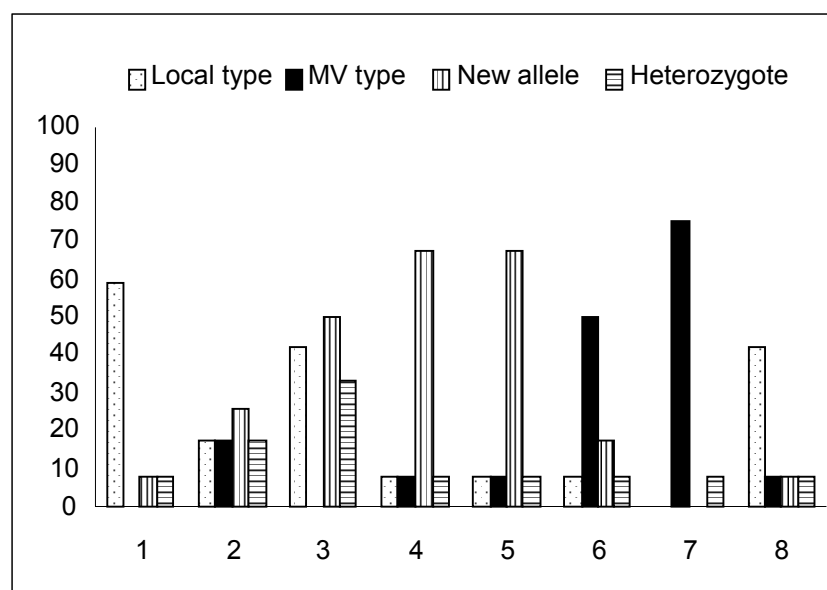
Among 12 SSR markers used in the study, 1–7 (8–59%) were observed polymorphic to their respective cross parents and produced the fragment (band) different from the ones produced by the respective cross parents. These fragments were unique to the progenies and were scored as new alleles. Based on these new alleles, the progenies of *Jethobudho/Pusa Basmati-1* were observed to be most diverse from their parents with highest (59%) new alleles (Figure 2a) and only 8% were shared with either parent and the rest were monomorphic. The cross parents—*Jethobudho* and *Pusa Basmati-1*, the traditional aromatic varieties of Nepal and India respectively with small to long grains, holding a special importance in grain production in Nepal for aroma—were, however, observed monomorphic for most loci examined. On the other extreme, the progenies of crosses *Biramphool/Himali*, *Thulogurdi*/NR10286 and *Ekle/Khumal-4* in Kaski and progenies of most crosses in Bara except *Mansara*/IR62161 were found to share most bands of local parents (42–64%) and these progenies shared only 8% of improved male parent used in the cross (Figure 2a and 2b). However, the progenies of *Naulomadhese*/IR36 in Kaski and *Mansara*/IR62161 in Bara showed the parental contribution mostly from the improved parents (75–80%) respectively. Besides, 8–25% of heterozygous was encountered in the PPB progenies of Kaski, and the maximum level of heterozygosity was revealed in *Thulogurdi*/NR10286 and *Ekle/Khumal-4*. Based on the allelic information, *Mansara/Khumal-4* progeny in Kaski was found most diverse in genetic composition with alleles shared with both the parents, and also comprised of new alleles different from parents and the heterozygous alleles (Figure 2a).



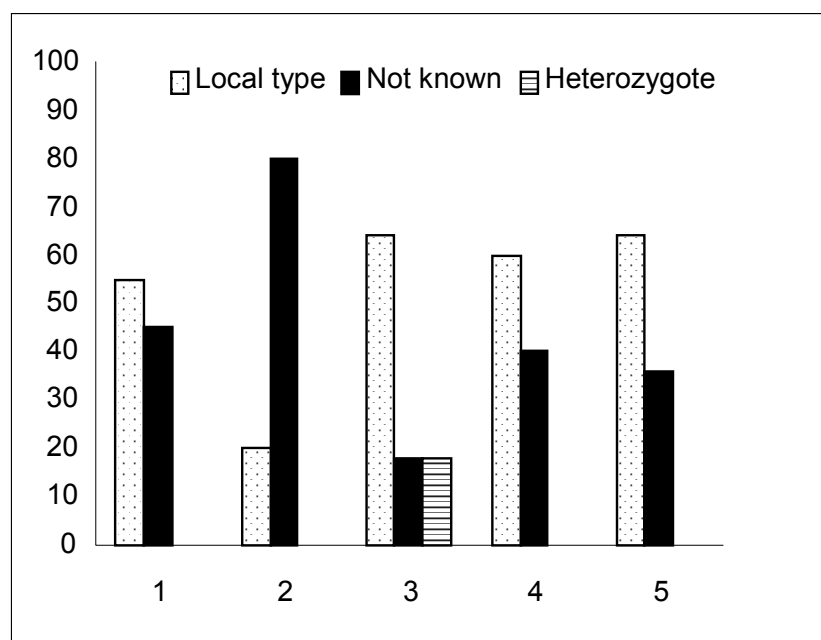
**Figure 1. Agarose gel electrophoresis of SSR products amplified using primers.**

(a): RM234 on PPB rice products of Kaski landraces. (From left to right: 1 *Biramphool* x *Himali*, 2 *Biramphool*, 3 *Himali*; 4 *Mansara* x *Khumal-4*, 5 *Mansara*, 6 *Khumal-4*; 7 *Thulogurdi* x *NR10286*, 8 *Thulogurdi*, 9 *NR10286*; 10 *Pusa basmati* x *Jethobudho*, 11 *Pusa basmati*, 12 *Jethobudho*; 13 *Pusa basmati* x *Jethobudho* EPD, 14 *Pusa basmati*, 15 *Jethobudho*; 16 *Sanogurdi* x *NR10285*, 17 *Sanogurdi*, 18 *NR10285*; 19 *Naulomadhese* x *IR36*, 20 *IR36*; 21 Blank (no *Naulomadhese*); 22 *Ekle* x *Khumal-4*, 23 *Ekle*, 24 *Khumal-4*)

(b): RM167 on PPB rice products of Bara landraces (From left to right: 1 *Dudhisaro* x *BG1442*, 2 *Dudhisaro*; 3 *Mansara* x *IR62161*, 4 *Mansara*; 5 *Lalka Basmati* x *IR62161*, 6 *Lalka Basmati*; 7 *Lajhi* x *Rampur Masuli*, 8 *Lajhi*; 9 *Lajhi* x *IR62161*, 10 *Lajhi*)



**Figure 2a. Percentage of parental contribution over the 8 PPB products of Kaski landraces detected by SSR markers distributed throughout rice genome. (Crosses: 1. *Biramphool*/*Himali*, 2. *Mansara*/*khumal-4* 3. *Thulogurdi*/*NR10286*, 4. *Pusa Basmati-1*/*Jethobudho*, 5. *Pusa Basmati-1*/*Jethobudho* EPD, 6. *Sanogurdi*/*NR10285*, 7. *Naulomadhese*/*IR36*, 8. *kle*/*Khumal-4*).**



**Figure 2b. Percentage of parental contribution over the 5 PPB products of Bara landraces detected by SSR markers distributed throughout rice genome. (Crosses: 1 *Dudhisaro*/BG1442, 2 *Mansara*/IR62161, 3 *Lalka Basmati*/IR62161, 4 *Lajhi/Rampur Masuli* and 5 *Lajhi*/IR62161 in series).**

Figures 3 and 4 show a diagrammatic representation of the genetic structure of PPB progenies of Kaski and Bara, respectively, and parental contribution of genetic constituents of respective parents to the progenies based on the alleles amplified by the SSR markers included in the study. Genetic similarity of each progeny was calculated for SSR data to show relationships to their respective parents. The maximum similarity index value obtained among the PPB progenies in Kaski was 0.88 for *Ekle/Khumal*-4 progeny, which indicated a close relationship with local parent *Ekle*. In Bara it was 0.72 between *Lalka Basmati*/IR62161 progeny and *Laka Basmati*, the local parent. The minimum similarity index values of 0.22 and 0.33 were obtained for *Sanogurdi*/NR10285 (Kaski progeny) and *Mansara*/IR62161 (Bara progeny) showing low contribution of local cross parents. However, the average genetic similarity coefficients obtained were 0.51 among 8 progenies in Kaski and 0.56 among 5 progenies in Bara, which is a reasonably good value, and indicated a level of genetic similarity with the local cross parents. These values could be interpreted as a result of genetic contributions of landraces to the PPB progenies that were detected by SSR markers.

In plant breeding programmes, the choice of parent varieties and information regarding the pattern of genetic variation and relationships and setting the objectives are crucial for effectiveness. In the present study, we carried out preliminary work to understand the diversity and genetic relationships of the progenies identified during the process of PPB in Bara and Kaski and to observe that the PPB process conserves local genetic resources by the use of SSR markers. Microsatellites (SSRs), sequence-tagged microsatellite site (STMS) or SSLP markers are PCR (polymerase chain reaction) based markers that are randomly distributed and are readily available in rice genome and have generated valuable information about genetic variation and relationships in rice (McCouch *et al.* 1997; Cho *et al.* 1997; Chen *et al.* 2000; Temnykh *et al.* 2000).

	Biramphool Himali	Mansara Khuma14	Thulo Gurdi NR10286	PB-1 Jethobudho	Sano Gurdi NR10285	Naulomadhese IR36	Ekle Khuma14	
1								RM5
2								RM213
3								RM232
4								RM226
5								RM164
7								RM234
8								RM223
9								RM206
9								RM242
11								RM224
11								RM167
12								RM247

**Figure 3. Diagrammatic sketch of the genetic structure of PPB products of Kaski showing parental contribution at the genetic level (see table 1 for PPB cross codes):**  
 local parent, improved parent, monomorphic, new allele, not known, no amplification and mono parents.

	Dudhisaro BG1442	Mansara IR62161	Lalka basmati IR62161	Lajhi Rampur masuli	Lajhi IR62161	
4						RM226
5						RM164
5						RM211
7						RM234
8						RM223
9						RM206
9						RM242
9						RM215
10						RM228
11						RM167
12						RM247

**Figure 4. Diagrammatic sketch of genetic structure of PPB products of Bara showing parental contribution at the genetic level (see Table 1 for PPB cross codes):**  
 local parent, not known, and no amplification.

For each primer evaluated, the maximum number of DNA segments amplified for rice genotype ranged from 1 to 3. Among 12 SSR loci, RM5, RM164, RM167, RM226, RM206, RM234, RM234, RM242 and RM247 showed some degree of polymorphism among the PPB progenies and their respective cross parents with the amplification of the allele (DNA fragment) different from the parents. This indicates that PPB populations are still segregating and to an extent it showed the diverse genotypes in farmers' fields with unique alleles different from the cross parents, possibly through intercrossing or mutation during the process of PPB and successive selection. *Jethobudho/Pusa Basmati-1* and *Mansara/Khumal-4* were the progenies detected as diverse in present molecular structure analysis that produced unique alleles across 2-7 SSR loci, and comprised of heterozygous alleles and shared the alleles of both parents. This SSR marker information could be employed in reflecting the genetic dissimilarity among the PPB progenies and the parents as these markers directly sample the DNA composition of the genomes (Prabhu *et al.* 1997).

On the other extreme, the progenies in Kaski *Biramphool/Himali*, *Thulogurdi/NR10286*, *Ekle/Khumal-4* and *Lalka Basmati/IR62161* in Bara found to share the alleles with their local parents at 5-7 SSR loci (Figures 2a and 3). This indicated an estimate of landrace contribution as local parent to respective progenies. Therefore, these progenies showed similarity to the landraces at the genetic level based on the SSR allelic data gathered. This could be explained that PPB has conserved the landraces at the gene level. However, the estimates of genetic similarity and dissimilarity and parental contribution in progenies depend on type of markers, location and number of markers employed and appropriate genome coverage of the sample (Bohn *et al.* 1999). Use of a combination of different markers, inclusions of a considerable random number of markers and right sampling are suggested for a better understanding of genetic structure analysis.

In the present study, it is interesting to note that the progeny *Mansara/Khumal-4* was very different from others in terms of number of unique bands, heterozygous alleles and the way of genetic contribution. The populations created diverse genotypes phenotypically in the field with options to select as *Mansara*, *Khumal-4* type with grain improvement and intermediate types (see Gyawali *et al.*, Volume II, p. 202). This progeny is genetically very different and desirable and could be used for improving the commonly grown varieties. The present study has employed only a few random SSR markers and preliminary observations were noted. To envisage and interpret the impact of PPB in detail, there is a need to study more markers covering the whole rice genome and therefore it is suggested that further studies be made to broaden the genetic base in breeding by combining the different marker systems.

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## On-farm evaluation of traits influencing outcrossing among rice (*Oryza sativa* L.) landraces of Central Terai, Kachorwa, Bara, Nepal

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### Abstract

Vast diversity of rice landraces is the major characteristic of the rice-production system in Nepal. Inter- and intrapopulation gene flow in rice landraces and with related wild and weedy species is one of the major sources of this diversity obtained from selection of escaping introgressed types. Cross-pollination is the most important component for gene flow. This study was carried out during the normal rice-growing season in Kachorwa, Bara to identify variation in traits that influence outcrossing in rice landraces and the relationships among them under on-farm conditions. Fourteen main season rice landraces were used in this study. Sample size was five for each trait studied. Primary tillers and primary panicles were used for this purpose. Landraces showing high intravarietal coefficient of variation (CV), so were a good source of a particular gene for future study. High intervarietal CV was found for stigma exertion (49.81%) followed by opening of lemma and palea (24.68%). However, angle of glume opening (7.55%) followed by pollen fertility (7.62%) and panicle length (8.69%) accounted for lower intervarietal CV. Well-exserted panicles were observed in all landraces. Wider angle of glume opening led to greater exertion of stigma as shown in their correlation (0.533,  $P < 0.050$ ). Correlation between opening duration of lemma and palea and pollen fertility ( $-0.516$ ,  $P < 0.059$ ) indicated a genotype with high pollen sterility had high demand for external pollen. Correlation between pollen fertility and spikelet fertility for non-bagged panicles ( $-0.502$ ,  $P < 0.068$ ) showed possibility of outcrossing in rice landraces. Since *Kariya Kamod* and *Lalka Basmati* had higher deficit in pollen, their good grain-filling characteristics for non-bagged panicles could only be supported by a high rate of outcrossing. This condition can also be explained by longer mean opening duration for lemma and palea on these landraces. These two fine and aromatic landraces fall under the same cluster of dendrogram, which should not be treated as totally self-pollinated ones, and special attention to their conservation is needed. Gene flow study on rice landraces under on-farm conditions is considered essential for better understanding of higher variation in landraces and to formulate an on-farm conservation strategy that is location and landrace specific.

**Key words:** Correlation, gene flow, landraces, outcrossing, *Oryza sativa* L., pollen fertility

### Introduction

Rice is economically, socially, and culturally important crop in Nepal. Therefore, it is the heart, head and stomach of Nepalese people. It occupies about 32% of agricultural area and production in Nepal (Maclean *et al.* 2002). A vast range of agroclimatic and sociocultural settings in the country give rise to a large number of rice landraces adapted to specific niches. Landraces are the major characteristic of rice-production systems in Nepal (Gauchan 1999).

Landraces are the heterogeneous crop populations that humans deliberately cultivate (Harlan 1975) and are not the product of modern plant breeding (Louette 2000). Farmers' varieties includes landraces and other varieties having no well-defined pedigree (Berthaud *et al.* 2001). Landraces are crop populations selected and maintained by farmers within the natural system of evolution (Allard 1999). More than natural selection, the selection imposed by farmers makes landraces significant with their social, cultural and religious ties in farming communities. Using molecular markers, Sharma and Leung (2002) and Bajracharya *et al.* (2004) concluded that Nepalese rice landraces may have a unique gene pool. Glaszmann (1986) also observed abundant rice varietal diversity along the foothills of the Himalayas,

and Khush (2000) considered the *Terai* region of Nepal, and Uttar Pradesh and Bihar of India is the primary centre for Asian aromatic rice. Migration (geneflow) is one of the important forces that maintain genetic diversity in plant populations (Oka 1974). Pollen-mediated geneflow is most important because this leads to introgression and recombination within the plant population. However, in the strict sense, outcrossing alone may not represent the total amount of geneflow experienced by a population (Govindaraju 1988).

In the case of rice, the outcrossing rate is varied over the species, and within varieties ranges from 0 to 50% (Oka 1988). The outcrossing rate of Nepalese rice landraces from mid-hill was observed as 0.00–4.19% in mid-hill conditions and 0.12–1.83% in inner *Terai* conditions (Amgain 2005).

The intravarietal genetic diversity in rice should be at a tolerable level for farmers to maintain the quality and yield; otherwise, the farmers start replacing such landraces (Amgain *et al.* 2004b). Since *ex situ* genebanks cannot conserve the landraces in a true sense, breeding strategies are the most important means of conserving landraces in the long run (Amgain *et al.* 2004a), and geneflow among rice landraces in particular conditions should be studied (Sthapit 1999; Amgain 2005). However, the geneflow in rice cannot be generalized and should be studied for specified landrace and location (Amgain 2005).

Outcrossing is highly influenced by reproductive biology and environmental conditions (Waines and Hegde 2003). The outcrossing rate on rice largely depends upon capacity of stigma to receive alien pollen and the disseminating ability of alien pollen grain (Oka 1988), and this ability is largely the function of traits that influence outcrossing. Thus, the traits that influence outcrossing have to be studied in local conditions. Therefore, this study was carried out to identify the variation in traits that influence outcrossing among rice landraces of Kachorwa, Bara (central Terai), Nepal under on-farm conditions, and their relationships during normal season.

### **Materials and method**

An on-farm study was carried out on 14 main season rice landraces (photoperiod sensitive) of central *Terai* (Kachorwa, Bara), Nepal during July–November 2003. The traits that influence outcrossing were studied during flowering season following the process described in IRRI (2002). Sample size was 5 for each trait studied. However, it was 10 for traits relating to grain length/breadth ratio. Observations were made on main tillers and primary panicles. Five primary panicles were bagged before anthesis to study spikelet fertility on bagged panicles while another five primary non-bagged panicles were also studied. The flag leaf character was studied as described below.

#### **Flag leaf character description**

Code	Description
001	Parallel with panicle and tip of flag leaf above panicle tip
002	Parallel with panicle and tip of flag leaf in equal position with panicle tip
003	Parallel with panicle and tip of flag leaf below panicle tip
004	Parallel with panicle and tip of flag leaf below/equal with panicle base
005	Away (>60°) with panicle and tip of flag leaf below panicle base
006	Drooping (>90°) with panicle and tip of flag leaf below panicle base



Duration of opening of lemma and palea on one individual spikelet/panicle from 5 primary panicles was monitored at 15-min intervals. About 20–25 florets from 5 random primary panicles were collected before anthesis and fixed in 70% ethanol. Pollen fertility was studied by extracting all anthers from 6 random spikelets collected. The anthers were gently crushed on a drop of 1% iodine-potassium iodide (IKI) and after 10 min the stained pollen was examined in 3 random microscopic fields under 40X magnification. The fertile pollen was counted as described by Virmani *et al.* (1997). A single population of a landrace was studied and all populations were pooled for correlation study. A dendrogram of rice landraces was constructed using Euclidean distance as a similarity measurement using average linkage method. MINITAB and SPSS for PC package were employed for statistical analysis.

## **Results and discussions**

### **Intravarietal variation in traits that influence outcrossing**

High coefficient of variation (CV) for flag leaf length, spikelet fertility on bagged panicles with higher mean for flag leaf breadth and lower mean for spikelet fertility on bagged panicles was found in *Aamaghauj*. Low mean spikelet fertility on bagged panicles despite good pollen fertility in this landrace may be due to lodging during flowering season. *Bhatthi* showed higher CV for flag leaf breadth and pollen fertility with higher mean value for spikelet fertility for bagged panicles, pollen fertility and stigma exertion with lower mean value on grain length/breadth ratio. *Dudhraj* showed high CV for grain length/breadth ratio and lower mean flag leaf length. Higher CV with lower mean value for stigma exertion and duration of opening of particular lemma and palea was found in *Harinker*. High CV for pollen fertility and low mean value was found in *Kariya Kamod*. It also showed high mean value for spikelet fertility on non-bagged panicles. *Lal Tenger* showed high CV for angle of glume opening, spikelet fertility for non-bagged panicles and for duration of opening of particular lemma and palea. Higher CV for grain length/breadth ratio was found in *Madhumala*. Higher CV for panicle length and for angle of glume opening with lower mean was found in *Pakher* (Table 1). This higher variation may be due to genetically heterogeneous population that was characteristic of landrace. Similar conclusions were made by Oka (1988) using square root of generalized variance for spikelet length and width.

Similarly, lower CV with wider mean angle of glume opening, flag leaf breadth, longer mean duration of opening of lemma and palea, and greater grain length/breadth ratio was found in *Pakher*, *Lal Tenger*, *Lalka Basmati* and *Kariya Kamod*, respectively (Table 1). Thus, these traits may have higher positive selective value for fitness in respective landrace populations. Since these traits were related to reproductive fitness for primarily seed-propagated rice species (Oka 1988), the correlation between farmers' selection and reproductive fitness during domestication plays a greater role during evolution of these rice landrace populations. However, the phenotypic plasticity for particular environment condition could not be neglected.

### **Intervarietal variation on traits influencing outcrossing**

High intervarietal CV was found for stigma exertion (49.81%), followed by duration of opening of lemma and palea (24.68%), flag leaf breadth (18.49%), flag leaf length (15.17%), spikelet fertility for bagged panicles (11.48%), grain length/breadth ratio (9.86%). Moreover, CV was lower for spikelet fertility in non-bagged panicles (3.7%), followed by angle of glume opening (7.55%), pollen fertility (7.62%), and panicle length (8.69%). Similar results for stigma exertion, spikelet fertility, and pollen fertility were observed by Joshi (2000) while studying some Nepalese rice landraces. Lower variation for spikelet fertility on non-bagged panicles and pollen fertility showed uniformity on grain setting among rice landraces.

Table 1. Mean, coefficient of variation and range of different traits influencing outcrossing among rice landraces, Kachorwa, Bara, Nepal.

Character	Muna	Jhalari	Syank-hole	Arbali Kodo	Seto Dalle	Kalo Jhyape	KukurKane	Okhale-1
Plant height, cm	Mean±SE	66.50±1.45	86.85±1.78	90.63±2.76	94.73±3.16	103.63±3.02	87.37±1.78	101.73±2.52
	CV %	11.51	10.50	16.68	17.06	15.97	11.21	13.62
	Range	48.00–84.00	71.00–105.00	48.00–114.00	65.00–119.00	53.00–129.00	62.00–103.00	75.00–125.00
Tiller no.	Mean±SE	1.70±.12	1.63±.13	1.10±.07	1.33±.11	1.20±.1	1.47±.13	1.10±.05
	CV %	41.31	42.20	37.09	46.51	45.91	49.79	27.74
	Range	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–2.00
Culm thickness, mm	Mean±SE	3.64±.18	3.45±.06	3.73±.15	3.44±.15	3.70±.13	3.68±.13	3.98±.10
	CV %	23.32	10.14	21.96	23.11	19.29	19.66	13.94
	Range	1.90–4.80	2.80–4.20	2.20–5.40	2.20–4.90	2.30–5.00	2.20–5.50	2.80–5.20
Leaf, no.	Mean±SE	13.70±.33	12.31±.52	11.45±.52	10.02±.54	13.79±.61	13.37±.38	11.59±.35
	CV %	13.57	21.65	24.77	27.96	23.92	15.65	16.40
	Range	10.00–17.00	5.00–17.00	4.00–18.00	5.00–17.00	8.00–20.00	8.00–17.00	8.00–15.00
Leaf sheath length, cm	Mean±SE	9.53±.21	11.41±.25	9.22±.32	9.57±.48	11.38±.5	9.73±.36	10.50±.3
	CV %	12.32	11.66	19.14	25.81	23.74	20.58	15.90
	Range	7.00–11.50	8.50–13.50	5.50–14.00	0.40–13.00	6.50–16.00	5.50–14.50	7.00–13.00
Leaf sheath width, cm	Mean±SE	0.52±.01	0.47±.01	0.48±.01	0.47±.02	0.48±.01	0.47±.02	0.50±.01
	CV %	17.78	17.51	17.96	26.80	20.01	23.50	20.00
	Range	0.30–0.70	0.30–0.70	0.30–0.70	0.30–0.80	0.30–0.70	0.30–0.70	0.30–0.80
Leaf blade length, cm	Mean±SE	34.20±1.21	28.20±1.03	31.88±.75	32.65±.96	34.03±1.34	28.67±.81	32.93±.84
	CV %	19.47	19.16	12.73	15.14	21.22	15.52	13.77
	Range	20.00–45.50	16.00–37.50	24.00–40.50	22.00–44.00	14.00–50.00	21.00–38.00	21.00–41.00
Leaf blade width, cm	Mean±SE	1.02±.01	0.93±.02	0.91±.02	0.91±.04	0.91±.02	0.92±.02	0.91±.02
	CV %	6.66	12.25	12.82	24.30	14.78	12.94	12.91
	Range	0.90–1.20	0.50–1.00	0.60–1.10	0.70–1.80	0.60–1.30	0.70–1.20	0.70–1.10
Flag leaf blade length, cm	Mean±SE	22.71±1.03	20.52±.81	26.22±1.04	22.94±1.27	24.41±1.2	19.53±1.02	22.52±1.08
	CV %	24.85	20.69	21.48	28.36	26.65	28.76	25.97
	Range	9.00–32.50	10.50–29.00	15.00–36.00	14–34.50	9.50–39.00	7.50–32.50	11.00–36.50
Flag leaf blade width, cm	Mean±SE	0.81±.02	0.68±.022	0.69±.02	0.74±.02	0.68±.02	0.73±.02	0.72±.02
	CV %	15.86	16.80	16.33	20.65	16.87	16.93	17.17
	Range	0.50–1.00	0.40–0.90	0.50–1.00	0.50–1.00	0.50–0.90	0.50–1.00	0.50–1.00

Table 1. (cont'd.)

Character	Muna	Jhalari	Syank-hole	Arbali Kodo	Seto Dalle	Kalo Jhyape	KukurKane	Okhale-1
Ear exertion, cm	Mean±SE	10.16±.71	8.19±.45	12.89±.72	14.41±.53	12.58±.6	12.02±.55	9.22±.77
	CV %	38.42	29.70	29.79	19.32	26.21	25.21	45.98
	Range	0.50–13.00	1.00–12.00	0.00–17.50	9.50–20.50	1.00–17.50	4.50–17.00	0.00–14.00
Finger length, cm	Mean±SE	5.13±.14	5.66±.13	5.16±.14	4.92±.18	8.45±.29	7.23±.20	9.72±.51
	CV %	14.95	12.96	15.16	19.71	18.81	15.77	28.78
	Range	3.50–7.00	4.00–7.00	3.50–6.50	3.00–7.00	5.00–11.00	5.00–10.50	4.50–15.00
Finger width, cm	Mean±SE	0.76±.03	0.95±.01	0.80±.02	0.65±.02	0.76±.02	0.80±.02	0.74±.02
	CV %	21.94	10.08	18.70	20.17	16.67	19.97	18.56
	Range	0.50–1.00	0.70–1.00	0.60–1.00	0.50–1.00	0.50–1.00	0.50–1.00	0.50–1.00
Finger no.	Mean±SE	6.70±.29	4.00±.17	4.60±.28	5.00±.24	5.17±.23	5.57±.23	4.20±.17
	CV %	22.60	22.57	24.37	25.30	24.27	22.96	22.89
	Range	4.00–10.00	2.00–5.00	3.00–7.00	3.00–7.00	3.00–8.00	3.00–9.00	1.00–6.00
Grains/spikelet, no.	Mean±SE	232.00±12.33	235.71±10.99	210.00±19.62	143.00±11.85	193.28±11.57	172.43±9.53	198.46±11.91
	CV %	27.62	24.67	36.20	42.29	32.24	30.30	31.76
	Range	145.00–380.00	111.00–370.00	71.00–300.00	39.00–246.00	66.00–358.00	87.00–299.00	100.00–320.00
Grain yield/plant, g	Mean±SE	5.13±.51	3.96±.36	2.04±.24	1.32±.22	2.70±.31	3.15±.44	2.43±.23
	CV %	52.24	58.27	46.26	85.33	62.14	76.99	50.26
	Range	1.10–10.96	0.96–8.42	0.72–3.99	0.21–5.01	0.21–6.95	0.67–10.84	0.38–4.62
Days to flowering	Mean±SE	74.87±.62	113.70±.47	119.31±1.16	126.67±.98	114.86±.56	115.70±.39	110.17±.69
	CV %	4.56	2.19	5.25	4.05	2.63	1.89	3.39
	Range	70.00–85.00	110.00–119.00	110.00–145.00	116.00–41.00	111.00–123.00	113.00–121.00	101.00–122.00
Days to maturity	Mean±SE	99.58±.69	152.82±.62	153.67±1.08	158.04±.52	156.21±.36	153.83±.34	143.38±.71
	CV %	3.54	2.17	2.74	1.62	1.27	1.22	2.68
	Range	93.00–107.00	142.00–159.00	144.00–157.00	151.00–62.00	151.00–159.00	149.00–157.00	140.00–151.00

The frequency of the flag leaf character was 0.143, 0.643, and 0.214 for flag leaf above panicle, flag leaf equal to panicle, and flag leaf below panicle respectively for landrace population. Full exertion of panicles from flag leaf sheath was found in all landrace populations showing that no gene for sheathed panicles had flowed from *Shatthi*. *Shatthi* is a rain-fed rice landrace with sheathed panicles and popularly grown in this locality (Amgain *et al.* 2004a). However, Yadav *et al.* (2003) reported different extent of panicle exertion for rice landraces of the same area, which may be due to the use of different rice landraces than ours.

### Correlation between different traits and outcrossing rate on rice landraces

The difference between the spikelet fertility on non-bagged panicles and bagged panicles does not truly represent the outcrossing rate of the rice landraces (Amgain 2005). Therefore, differences shown in Table 2 are not truly the differences in outcrossing rates of rice landraces. The microenvironment created by a bag may also contribute to this difference. However, the correlation between pollen fertility and spikelet fertility on bagged panicles (0.125,  $P < 0.671$ ), and between pollen fertility and spikelet fertility on non-bagged panicles (-0.502,  $P < 0.068$ ) indicated that there should have been some extent of outcrossing in rice landrace populations. However, Amgain (2005) obtained an outcrossing rate that had non-significant correlation with spikelet fertilities for bagged and non-bagged panicles. High spikelet fertility on non-bagged panicles with low pollen fertility can only be possible by outcrossing, despite their positive correlation in control conditions as obtained by Devanand *et al.* (2000). Higher pollen sterility for *Kariya Kamod* (27.44%) followed by *Lalka Basmati* (11.14%) showed there must be high outcrossing rate in these two landraces showing higher spikelet fertility on non-bagged panicles.

Results from Amgain *et al.* (2004b) also support this; they observed higher proportion of hybrid swarm in *Lalka Basmati* population at the same location. High outcrossing might have led the *Lalka Basmati* population to show many introgressed types. However, the introgressed types could easily be detected and could be selected out by farmers in *Kariya Kamod*, showing its low proportion.

The correlation between stigma exertion and spikelet fertility for bagged panicles (0.738,  $P < 0.003$ ) showed selfing is possible even in exerted stigma. However, Ramalingam and Rangaswamy (1997) found that stigma exertion has a direct relationship with outcrossing in Cytoplasmic Male Sterile (CMS) lines.

**Table 2. Difference between the mean spikelet fertilities for bagged and non-bagged panicles of rice landrace.**

Landrace	Mean spikelet fertility (%)		Difference (%)	Mean pollen fertility (%)
	Non-bagged panicles	Bagged panicles		
Aamaghauj	86.72	57.07	29.65	95.02
Anga	88.78	76.71	12.07	98.55
Bhatthi	88.12	85.88	2.25	99.62
Dudharaj	90.08	79.78	10.30	98.91
Harinker	91.91	67.49	24.42	99.22
Jagar Nathiya	89.26	71.17	18.09	99.2
Kariya Kamod	94.32	66.82	27.50	72.56
Karma	93.92	73.10	20.81	90.58
Khera	88.85	87.90	0.95	96.80
Lal Tenger	82.35	66.96	15.39	98.29
Lalka Basmati	93.55	69.13	24.42	88.86
Madhumala	93.33	74.37	18.96	96.92
Pakher	91.22	68.39	22.83	98.97
Parewa Pankha	87.59	65.60	21.99	93.92

The flag leaf breadth showed significant negative correlation with spikelet fertility for non-bagged panicles while it was significantly positive for flag leaf character (Table 3). This indicates that the flag leaf may obstruct the free movement of pollen reducing chances of outcrossing. The correlation between flag leaf character and flag leaf breadth ( $-0.601$ ,  $P < 0.23$ ) also showed the narrower flag leaf may promote outcrossing. Virmani and Edwards (1983) also considered that narrower flag leaf promotes outcrossing in rice. Narrower flag leaf was observed in *Kariya Kamod* and *Lalka Basmati* (Table 1).

**Table 3. Correlation between different outcrossing influencing traits among rice landraces.**

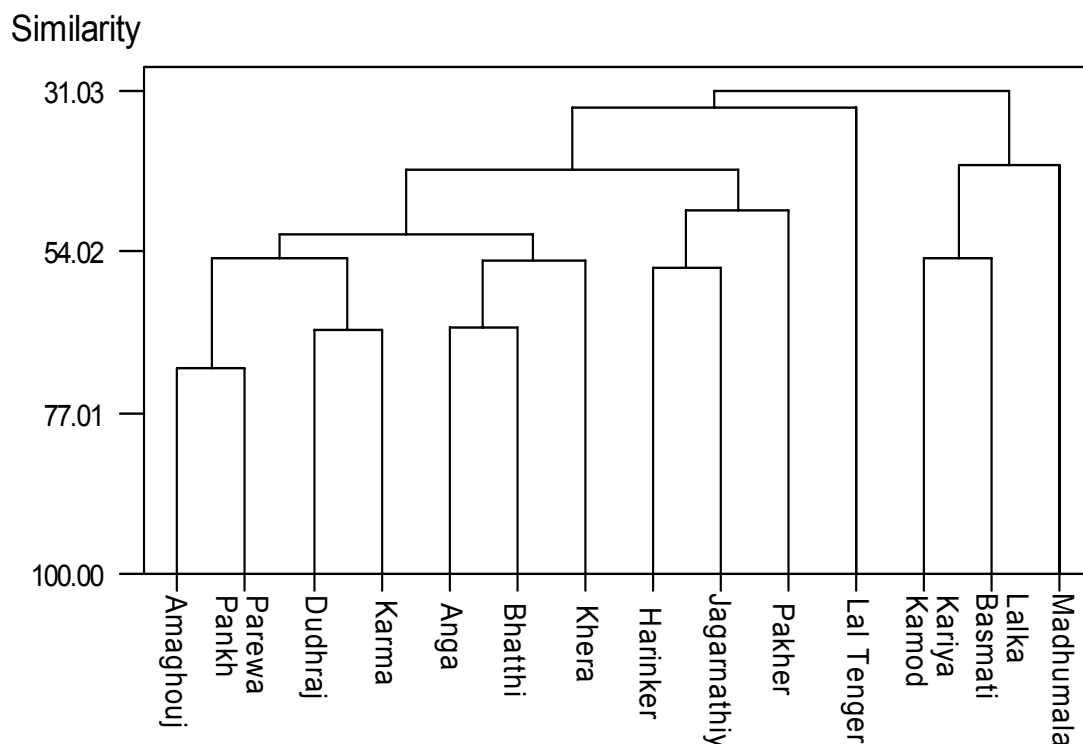
	Flag leaf length	Flag leaf breadth	Stigma exertion	Spikelet fertility (non-bagged panicles)	Pollen fertility	Spikelet fertility (bagged panicles)	Duration of opening of lemma and palea	Panicle length	Grain length/breadth ratio	Flag leaf character
Angle of glume opening	-0.091	-0.076	0.533*	-0.304	-0.001	0.427	0.119	-0.428	-0.017	-0.171
Flag leaf length		-0.117	0.370	-0.134	-0.229	-0.073	-0.128	0.455	0.439	-0.120
Flag leaf breadth			-0.217	-0.666**	0.500	-0.045	-0.297	0.119	-0.447	-0.601*
Stigma exertion				0.017	-0.203	0.738**	0.531	-0.153	0.049	0.134
Spikelet fertility (non-bagged panicles)					-0.502	0.073	0.437	0.274	0.299	0.583*
Pollen fertility						0.125	-0.516	-0.248	-0.740**	-0.578*
Spikelet fertility (bagged panicles)							0.398	-0.183	-0.341	0.122
Duration of opening of lemma and palea								-0.032	0.234	0.384
Panicle length									0.368	0.054
Grain length/breadth ratio										0.335

\*, \*\* significant at 5% and 1% level of significance respectively.

The correlation between duration of opening of lemma and palea and pollen fertility ( $-0.516$ ,  $P < 0.059$ ) showed the genotype with high pollen sterility has high demand for external pollen; thus lemma and palea remain open for longer duration. Wider angle of glume opening also led to greater exertion of stigma as shown by their correlation ( $0.533$ ,  $P < 0.050$ ). A similar result of significant positive correlation for outcrossing rate with angle of glume opening and duration of opening was detected by Singh *et al.* (2003). Amgain (2005) also found angle of glume opening was positively correlated with outcrossing rate in Nepalese rice landraces. However, he obtained shorter duration for glume opening in rice landraces of mid-hill of Nepal than in our result. The difference may be due to genotype as well as environmental differences.

The pollen fertility was also negatively correlated with the grain length/breadth ratio ( $-0.578$ ,  $P < 0.030$ ) having high mean value with low variation in *Kariya Kamod* (3.38) and *Lalka Basmati* (3.33). Thus, these landraces were deficit in pollen and need outcrossing to give good grain-filling in the non-bagged panicles, which was supported by correlation between spikelet for non-bagged panicle and pollen fertility. Longer mean duration for opening lemma and palea also supports it (Table 1). High grain length/breadth ratio seems to be associated with high stigma exertion (Li and Chen 1985) which promoted outcrossing.

Cluster analysis using all studied traits influencing outcrossing also cluster these two landraces into the same group (Figure 1). These two aromatic rice landraces were highly demanded by market.



**Figure 1. Dendrogram showing the relationship among rice landraces based on traits influencing outcrossing.**

Quality and aroma are the main preferences and identity of these landraces that was highly influenced by intrapopulation variability leading to quality degradation. Outcrossing increases the intrapopulation variability; however, higher intrapopulation variability may degrade the quality of the rice product as well as its yield and is not desired by the farmers. Then, farmers start replacing the particular genotype (Amgain *et al.* 2004b). However, outcrossing provides variability in landraces upon which farmers' selection leads to new population types. Thus, the intrapopulation variability should be at a tolerable level in its social, economic and biological aspects.

### Conclusion

Any conservation strategy for a particular crop is influenced greatly by its reproductive biology, which impacts on its genetic structure over the years. The rice landraces of Kachorwa, Bara showed higher variation in their floral biology, which required special attention for the conservation of the landrace diversity with respect to floral biology of rice landraces. Owing to ample possibility of outcrossing in some rice landraces, treating them as totally self-pollinated will mislead any conservation strategy. Special attention should be given to fine and aromatic rice landraces *Kariya Kamod* and *Lalka Basmati*. Thus, gene flow study on rice landraces in on-farm conditions is essential to formulate on-farm conservation methodologies that are location and landrace specific. However, a balance between varietal diversity and farmers' selection with respect to social, biological and economic aspects of rice landraces should be made.

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## Landrace-specific agronomic practices of rice (*Oryza sativa* L.) in Bara, Nepal

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### Abstract

Rice (*Oryza sativa* L.) in Nepal is the number one staple food crop covering 1.5 million ha with average productivity of 2745 kg/ha. It plays a pivotal role in the economy and livelihoods of the vast majority of Nepalese people. Substantial *in situ* conservation of diverse landraces of rice in many diverse ecosites of Nepal has continued since ancient times. Key informant surveys were carried out during rice seasons in 2003–04 in Kachorwa, Bara in order to document different types of rice landraces and their specific agronomic practices in on-farm conservation along with their socioeconomic and cultural values. Three different production systems of rice landraces are being practised in Bara district of Nepal: *Bhadaiya* (monsoon-season early rice), *Aghani* (full-season late rice) and *Ghol* rice (deepwater, full-season late rice). The overall seasonal area coverage by *Bhadaiya*, *Aghani* and *Ghol* rice was found to be 50, 20 and 5% respectively. Both *Bhadaiya* and *Aghani* are transplanted rice paddies whereas *Ghol* rice is direct seeded. The predominant cropping patterns under each rice culture, types of landraces, their cultural practices, grain and straw yield ranges, quality traits and price values in the local markets along with their socioeconomic and cultural values are presented. *Lalka Basmati* and *Kariya Kamod* are high-quality *Aghani* landraces that are aromatic with high values, whereas among *Bhadaiya* rice *Khera* and *Sathi* are religiously and culturally valuable landraces, *Mutmur* and *Nakhisaro* taste very good and are grown with low inputs and *Sotwa* is also good in taste and easily digestible as well. *Bhatti* and *Silhat* are two deepwater rice types that have been grown as monoculture for a long time despite their coarser grain quality and lower yields. Documentation of such studies in other sites is also suggested. In addition, soil fertility management, disease and pest management studies are required to improve and sustain the productivity levels of different promising landraces of rice. Postharvest and market promotional activities also seem necessary in the near future.

**Key words:** Agronomic practices, landraces, rice, Nepal

### Introduction

Rice (*Oryza sativa* L.) in Nepal is the number staple food crop covering 1.5 million ha with a total annual production of 4.2 million tons and average productivity of 2745 kg/ha (MOAC 2002). It plays a pivotal role in the economy and livelihoods of the majority of the Nepalese people. Nepalese farmers have been growing many and diverse kinds of rice landraces since ancient times. *In situ* conservation on-farm of diverse landraces of rice has been practised by farmers for years in many diverse ecosystems, ranging from plain *Terai* to the high hills of Nepal. As many as 63 and 33 different rice landraces in Kaski and Bara ecosites, respectively (ABD 1999) were reported being grown by the farmers.

There are three different categories or production systems of rice landraces found in Bara: *Bhadaiya*, *Aghani* and *Ghol* rice. Rice cultivation practices vary according to their types, land types and soil fertility status where they are grown including the availability of water (Basnet and Joshi 1992; Joshi *et al.* 2001). Socioeconomic reasons for *in situ* conservation of rice landraces in Bara have been highlighted by Gauchan (1999). Farmers' preferences of these landraces to one another with respect to their traits and use values are important for their livelihoods.

Nevertheless, there still exists a gap in our knowledge of the types of rice culture and their productivity in terms of grain and straw yields, including agronomic management practices of landraces. It is necessary to document landrace-specific agronomic practices of on-farm conservation of these large numbers of landraces in addition to their socioeconomic and

cultural values. Such information will help identify their distinctness in terms of genetic bases, utility values and specific management practices. Therefore, key informant surveys were carried out at Kachorwa, Bara during the rice growing seasons in 2003–2004 in order to document different types of rice culture, their husbandry practices, cropping patterns, landrace-specific socioeconomic and cultural values and the storage systems.

### Materials and methods

Key informant surveys of rice farmers were done at Kachorwa VDC in Bara district in 2003 and 2004. Four key informant groups were randomly selected from different wards (1, 2, 3, 4, 5 and 6) in each year to participate in the group discussion and to obtain answers for the predesigned questionnaires related to crop management practices for different landraces of rice. A total of 80 farmers participated in the group discussion and each group had mixed representation of both men and women farmers. The information collected was critically reviewed, processed and screened for their mean values in report writing.

### Results and discussion

The synthesis of the key information survey revealed that among total rice acreage, rain-fed rice area is predominant (46.3%), followed by irrigated rice (35%), upland rice area (13.7% and *Ghol* rice (5%) in the Kachorwa VDC. Three different production systems of rice culture—*Bhaidiya*, *Aghani* and *Ghol* rice—are practised by the farmers in Kachorwa. On an overall basis, coverage by *Bhaidiya* landraces is dominant in 50% of the total rice acreage followed by *Aghani* rice landraces (20% of the total rice acreage) (Table 1). However, there has been no replacement of *Ghol* rice landraces so far and farmers are growing only two types of cultivars such as *Bhatti* or *Silhat* landraces. *Ghol* acreage is at a very minimal level (5% of total rice acreage).

**Table 1. Bhaidiya, Aghani and Ghol rice-based cropping patterns at Kachorwa, Bara, 2003/04.**

<b>Bhaidiya Rice</b> (Landraces in 50% of total rice area)		<b>Aghani Rice</b> (Landraces in 20% of total rice area)		<b>Ghol Rice</b> (Landraces in 5% of total rice area)	
<b>Cropping pattern</b>	<b>Coverage (%)</b>	<b>Cropping pattern</b>	<b>Coverage (%)</b>	<b>Cropping pattern</b>	<b>Coverage (%)</b>
1. Rice–Wheat	25–50	1. Rice–Wheat	25–60	1. Rice–Fallow	95–98
2. Rice–Potato	20–30	2. Rice/Lentil	20–50	2. Rice–Mungbean	02–05
3. Rice/Lentil–Fallow	15–80	3. Rice–Maize	18–20		
4. Rice–Maize (winter)	10–30				
5. Rice–Vegetables–Maize (spring)	10–25				
6. Rice–Rapeseed	10–20				

The cropping patterns under different rice cultures were observed (Table 1). The predominant cropping patterns in *Bhaidiya* rice were rice–wheat, rice–potato, rice/lentil, rice–maize (winter), rice–vegetables – maize (spring) and rice–rapeseed (Toria) and in *Aghani* rice were rice–wheat, rice/lentil and rice–winter maize. Predominant cropping in *Ghol* rice was rice–fallow, although mungbean has been introduced in such areas to some limited extent in recent years.

### Important landraces

All *Bhaidiya* and *Aghani* types are grown as transplanted rice with dry nursery seed-beds, whereas *Ghol* rice landraces are direct seeded rice grown in deep water that inundates after the start of the monsoon rain. Important cropping patterns under *Bhaidiya* rice based cropping patterns are rice–wheat (with 25–50% coverage), rice–potato (20–30%), rice/lentil–fallow (15–80%), rice–winter maize (10–30%), rice–vegetables–winter maize (10–25%) and

rice-rapeseed (10–20%). Major cropping patterns under *Aghani* rice-based system are rice–wheat sequential cropping with an average of 25–60% coverage, followed by rice/lentil (relay cropping) and rice–maize having the average coverage of 20–50% and 18–20%, respectively. The cropping pattern under *Ghol* rice is rice–fallow having the lions' share of 95–98% coverage. However, the short-season mungbean crop introduced in recent years covers about 2–5% area of the *Ghol* rice acreage.

Important landraces under *Bhadaiya*, *Aghani* and *Ghol* rice types, their quality traits and grain and straw yield range maintained at Kachorwa, Bara are shown in Table 2. Despite lower yield trends, these cultivars are valued by the farmers for their qualitative characters as well as straw yield that is valuable for feeding livestock and making dung-cakes for fuel purposes.

**Table 2. Important landraces under *Aghani*, *Bhaidya* and *Ghol* rice types, their quality traits and grain and straw yield range at Kachorwa, Bara, 2003/04.**

Landraces	Quality traits valued for conservation	Grain yield (t/ha)	Straw yield (t/ha)	Price of rice grain (Rs/kg)
<b>Bhaidya Rice (T)</b>				
1. Mutmur	Medium grain, tasty, contentment feeling after eating, rain-fed rice, low fertility respondent, robust seed/seedlings.	2.4–3.6	4.0–6.0	8.0–9.0
2. Nakhisaro	Medium grain, soft and tasty, less insect and disease problem, good response in low input.	2.4–3.0	4.0–6.0	7.0–8.0
3. Dudhisaro	Medium	2.4–3.0	4.0–6.0	7.0–8.0
4. Sathi	Coarse grain, religious and cultural values (Chhath Parwa), good price, quality rice pudding.	0.6–2.4	2.4–4.5	12.0–14.0
5. Sotwa	Coarse grain, soft, tasty, medicinal value (could be taken while in fever and easily digestible soft diet prepared for cholera patients as well), fewer insect and disease problems.	1.8–3.0	5.0–6.5	7.5–8.0
6. Rago	Coarse grain	1.8–3.0	3.0–4.0	6.0–7.0
<b>Aghani Rice (T)</b>				
Lalka Basmati (RT)	Aromatic, fine grain and tasty, high price, high cultural values and good for rice pudding	1.8–3.0	6.0–7.5	21.0–22.0
Kariya Kamod (RT)	Aromatic, fine grain and tasty, high price, high cultural values and quality rice pudding	1.5–2.4	6.0–8.0	21.0–22.0
3. Mansara	Medium grain	1.5–1.8	5.0–6.0	8.0–9.0
4. Jagarnathya	Medium grain	1.5–2.4	6.0–8.5	8.0–9.0
5. Laltengar	Medium grain	1.5–1.8	6.0–8.0	8.0–9.0
6. Harinker	Medium grain	2.0–3.0	4.5–6.0	8.0–9.0
7. Anga	Medium grain	1.5–1.8	2.5–3.5	8.0–9.0
8. Khera	Coarse grain, religious and cultural values (necessary for Karik Maharaj and Govinda Puja)	1.8–3.6	5.0–6.0	9.0–10.0
9. Laki	Coarse grain	2.4–3.0	6.0–8.5	8.0–9.0
10. Karma	Coarse grain	2.4–3.6	6.0–8.0	8.0–9.0
<b>Ghol or Deep Water rice (DS)</b>				
1. Bhatti	Coarse grain	1.5–2.4	2.5–3.0	7.50
2. Silhat	Coarse grain	1.5–2.4	2.5–3.0	7.50–8.0

RT, T, and DS refer to re-transplanted, transplanted, and direct seeded rice types.

There are six landraces in *Bhadaiya* types (Figure 1) of which three are medium-quality rice (*Mutmur*, *Nakhisaro* and *Dudhisaro*) and three are coarse grain types (*Sathi*, *Sotwa* and *Rago*). Landraces like *Sathi* and *Khera* are highly valuable from a religious point of view. There are 10 landraces under *Aghani* types (Figure 2) of which two are aromatic and fine types (*Lalka Basmati* and *Kariya Kamod*), five medium-quality landraces (*Mansara*, *Jagarnathya*, *Laltengar*, *Harinker* and *Anga*) and three coarse-grain types (*Khera*, *Lajhi* and *Karma*). Only two types of landraces are being grown under the category of *Ghol* rice culture (*Bhatti* and *Silhat* with 1.5–3.0 t/ha grain yield and 2.5–3.0 t/ha straw yield). The highest price for the rice

(Rs.21–22/kg) was fetched by *Aghani* type such as *Lalka Basmati* and *Kariya Kamod* for their fine and aromatic qualities followed by coarse grain *Khera* (Rs.9-10/kg) owing to its cultural value for *Karik Maharaj* and *Govinda Puja* (worship). However, among *Bhaidya* types, *Sathi* fetches the price of Rs. 12.0-14.0/kg because of its religious value for *Chhath*. Also, these landraces have a well-established local market at Kachorwa.

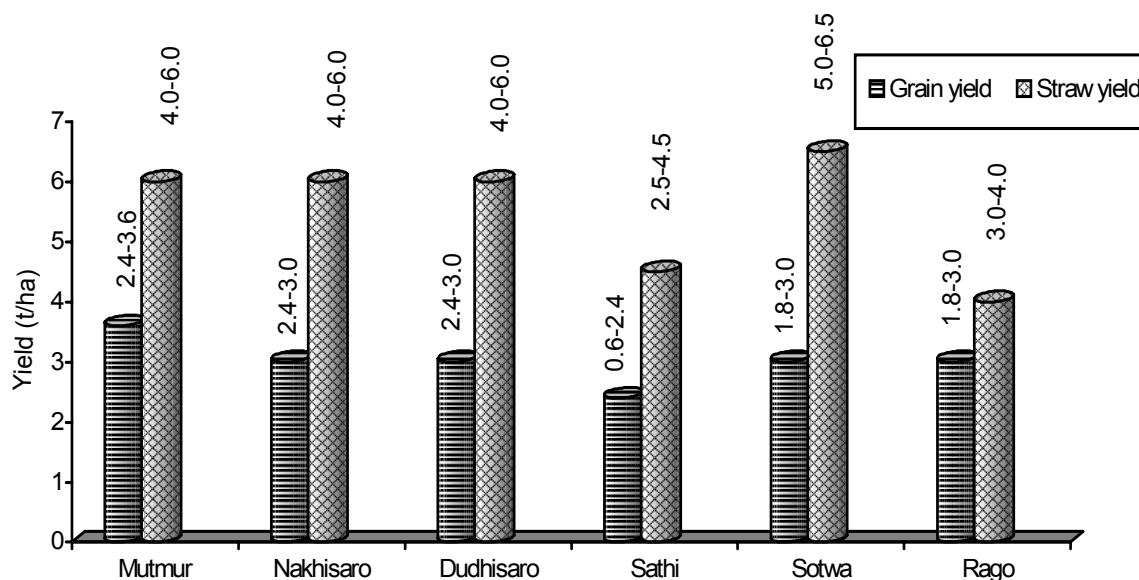


Figure 1. Average grain and straw yield of *Bhaidiya* landraces.

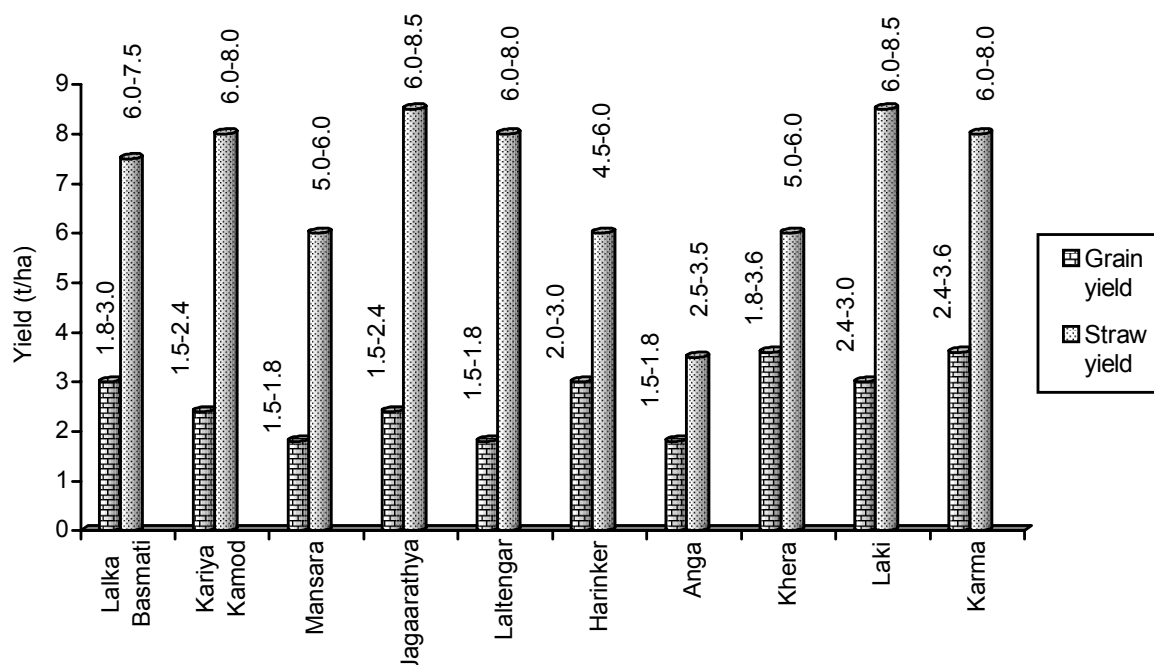


Figure 2. Average grain and straw yields of *Aghani* landraces.

The landraces are unique in their qualitative characters of the grains and quantitative straw yields, which are important for maintaining stall-fed livestock by the farmers. Besides, selling of rice straw at the village level is not a problem if it is stored for a few months after rice threshing. Rice straw is used to make thatch roofs and warm thatch houses, straw mats and other attractive items of local importance and use. Some storage structures are based on the use of rice straw. Cow-dung cakes are prepared by mixing cow dung with rice chaffs (by-products) in a certain proportion, which is sun-dried for a week or so. Dung-cakes are used for fuel for about 6–7 months from October to April. This is a very common practice in the *Terai* of Nepal that determines the use of manure from fertilizing the croplands. The religious and aesthetic significance of various landraces such as *Khera*, *Mutmur*, *Nakhisaro*, *Sathi* and *Sotwa* is also reported by Rana *et al.* (2000).

### **Agronomic practices**

The farmers for different rice cultures follow different cultural practices (Table 3). *Bhadaiya* and *Aghani* landraces are totally managed as transplanted rice culture whereas direct seeding is practised for *Ghol* rice. Dry nursery bed is the most common practice followed in *Bhadaiya* and *Aghani* rice, but for *Ghol* rice, direct seeding is practised. Land preparation for transplanted rice is done by ploughing dry fields 3–5 times by bullock-drawn ploughs early in the season followed by 1–2 wet ploughing and puddling during transplanting. But, in the case of *Ghol* rice, ploughing of dry fields is done 4–5 times by bullock-drawn ploughs and direct seeding of rice is followed by final planking to cover the seeds.

Transplanting for *Bhadaiya* and *Aghani* is done during June–July, direct seeding of *Ghol* rice from 15 to 30 May. Onset of pre-monsoon rains is necessary for both transplanting and direct seeding of rice. There is an occasional practice of *Kharahwan* planting only in the case of two types of landraces (*Basmati* and *Kariya Kamod*) in *Aghani* rice. *Kharahwan* planting means where established rice hills that were transplanted earlier are either partially or fully uprooted 35–40 days after transplanting, then re-transplanted. Half of the tillers from each mother (or transplanted) hills from the main rice field are used as *Kharahwan* by re-transplanting of 3–5 *Kharahwan*/hill in the newly puddled field. The rest of the seedlings are planted as *Kharahwan* in the main transplanted plots. This practice helps ensure flood disaster management of the inundated rice fields to some extent as well as fills gaps in the hills during crop establishment caused by various factors.

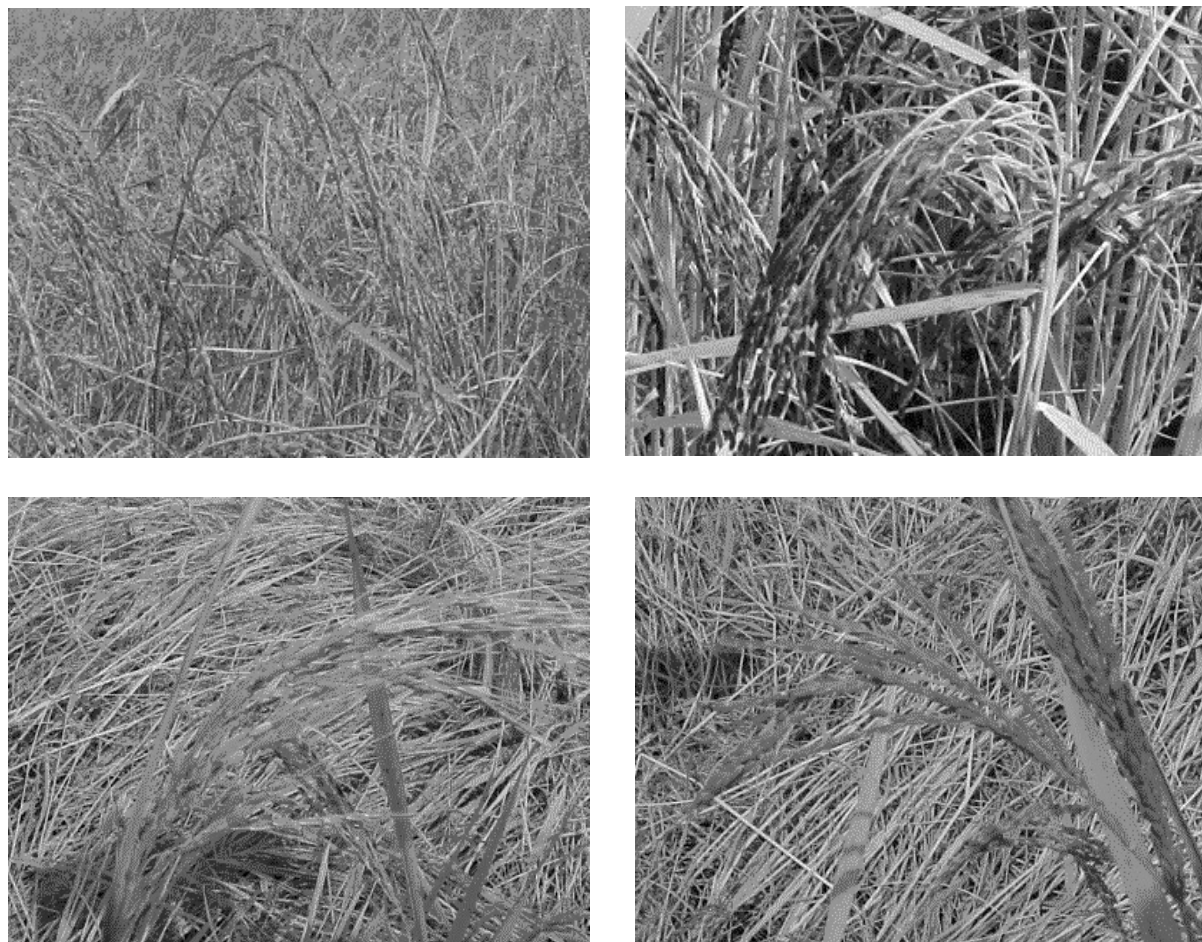
Maturity days vary among different rice cultures. This also influences the subsequent cropping patterns. *Bhadaiya* rice is early maturing, followed by *Aghani* rice and *Ghol* rice, which has the longest duration. Figure 3 shows *Lalka Basmati*, *Kariya Kamod*, *Khera* and *Bhatti* at maturity stage. Farmers apply the largest amount of fertilizer in *Bhadaiya* followed by *Aghani* and *Ghol* rice. *Ghol* rice is not weeded because of constant water stagnation in the growing fields. Two hand-weedings each at 30 and 60 days after transplanting are practised in all transplanted rice cultures. Details of all cultural practices are given in Table 3. Threshing and cleaning of rice bundles are mainly done manually or by tractors. Manually threshed straw bundles are re-threshed by allowing bullocks to walk over the straw piled around a pivotal pole or by the tractor. Then, cleaning of the threshed rice is done to remove dust and straw particles by using *Nanglo* (a round woven bamboo tray), or manually or power-operated winnowing-fans.

**Table 3. Different agronomic practices for different rice cultures, 2003/04.**

Practices	<i>Bhadaiya</i> rice	<i>Aghani</i> rice	<i>Ghol</i> rice
Nursery type	Dry nursery	Dry nursery	Direct seeded (DS) rice
Seed rate (kg/ha)	55–60	55–60	80–90
Seedling age (days) during transplanting	25–35	30–40	Direct seeded
Kharahwan planting (Re-transplanting)	–	Limited to two landraces: <i>Basmati</i> and <i>Kariya Kamod</i>	–
Seeding/transplanting period	1 <sup>st</sup> week of June to 1 <sup>st</sup> week of July depending on rains	3 <sup>rd</sup> week of June to 2 <sup>nd</sup> week of July depending on rains	DS within 4 <sup>th</sup> week of May to 1 <sup>st</sup> week of June depending on pre-monsoon rains
No. plants/hill	3–5 transplanted	3–5 transplanted	–
Spacing (cm x cm)	25 to 30 hills/m <sup>2</sup>	35–50 hills/m <sup>2</sup>	Direct seed broadcast
Fertilization <sup>†</sup>	Urea (90–120 kg), DAP (60 kg), MOP (40–60 kg) and FYM 5–6 t/ha. All the nutrients and half of the urea applied as a basal application with the remaining half of the urea top-dressed soon after the first weeding.	Urea (60–120 kg), DAP (60–90 kg) and FYM 5–6 t/ha. All the nutrients and half of the urea applied as a basal application with the remaining half of the urea top-dressed soon after the first weeding.	Urea (0–30 kg) and DAP (0–50 kg) per ha all applied as a basal application.
Weed control <sup>‡</sup>	Two hand-weedings each at about 30 and 60 days after transplanting	Two hand-weedings each at about 30 and 60 days after transplanting	–
Insect control	Usually, no insect damage observed beyond ETL, but severe gundhi-bug attacks used to happen in some years and Metacid-50 was sprayed during grain-filling stage, if infested.	No insect damage observed beyond economic threshold level (ETL).	Insect damage not so keenly observed but less problematic so far.
Disease control	Brown-spot Khaira disease reported by some farmers.	Brown-spot Khaira disease reported by some farmers.	Severe brown-spot disease reported but no control measures followed so far.
Days to maturity	100–120 days	150–160 days	200–210 days
Harvesting period and method	Oct. 1–30; Manual harvesting with sickle	Nov. 1 to Dec. 15; Manual harvesting with sickle	Dec. 1–15; Manual harvesting with sickle
Threshing and cleaning	Manual threshing followed by moving of bullocks over straw or by tractors. Then, rice paddies are manually cleaned to remove dust and straw particles by using Nanglo (a bamboo tray woven in circular shape) or by manually operated winnowing-fans.	Manual threshing followed by moving of bullocks over straw or by tractors. Then, rice paddies are manually cleaned to remove dust and straw particles by using Nanglo or by manually operated winnowing-fans.	Manual threshing followed by moving of bullocks over straw or by tractors. Then, rice paddies are manually cleaned to remove dust and straw particles by using Nanglo or by manually operated winnowing-fans.

<sup>†</sup> A limited number of farmers used zinc sulphate at 15–30 kg/ha and muriate of potash at 40–60 kg/ha for both *Bhadaiya* and *Aghani*.

<sup>‡</sup> One spraying of Butachlor within 3–5 days after transplanting was the weed control method adopted by some elite farmers.



**Figure 3. *Lalka Basmati* (top left), *Kariya Kamod* (top right), *Khera* (bottom left) and *Bhatti* (bottom right).**

Depending upon the volume of rice to store, different storage methods have been practised by the farmers. Threshing is done soon after proper drying of the rice. Rice is stored in plastic bags, gunny sacks, big earthen pots, earthen storage structures (*Beris* or *Kothis*), netted bamboo mats called *Bhakaris*, and drums. Dried straw is carefully piled together in the heap to store them for protection from the dampness or moisture. Baniya *et al.* (1999) have reported different seed storage systems of rice in the *Terai* and the mid-hills at household level in Nepal. Irrespective of the landraces or recommended rice varieties, due care in storage is given to protect from rice moths and rats by the farmers at large.

Some farmers rear cats to watch against storage rats and such a system of biological control of rats appears to be very much effective and sustainable.

### **Conclusion**

Many important rice landraces are cultivated by the farmers at Kachorwa, Bara. The cultural practices differ according to their types and existing growing conditions. There seems to be ample scope to improve current rice landrace management practices by documenting the farmers' knowledge base thereby utilizing them for further improvement of the yield levels. The knowledge can also be used to promote fine-quality landraces like *Lalka Basmati* and *Kariya Kamod* and some other culturally valuable ones (*Khera*, *Mutmur*, *Nakhisaro*, *Sathi* and *Sotwa*) for commercial production and marketing. *Lalka Basmati* and *Kariya Kamod* are aromatic landraces with high values grown in *Aghani* season, whereas among *Bhadaiya* rice

*Khera* and *Sathi* are religiously and culturally valuable landraces. *Mutmur* and *Nakhisaro* are good in taste and are grown with low inputs and *Sotwa* is tasty and has special value, which could be taken during the time of fever and is easily digestible. *Bhatti* and *Silhat* are two deepwater rice types that have long been grown as monoculture despite their coarse grain quality and low yields. Such traits would substantially contribute to the sustainability of *in situ* conservation of important rice landraces in Bara and in the vicinity having similar agroecosystems and socioeconomic conditions. Documentation of such studies in other sites is also suggested. Further work on improving the production and productivity under each type of landrace production system from current levels including soil fertility (zinc application), disease and pest management aspects, and postharvest and market promotional activities seems necessary to ultimately scale-up their commercialization in the near future.

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## Finger millet

**Stability and adaptation of finger millet (*Eleusine coracana* (L.) Gaertn.) landraces**

Bal K. Joshi, Radha K. Tiwari, Resham B. Amgain, Pitamber Shrestha, Sanjaya Gyawali, Ashok Mudwari and Bimal K. Baniya

**Abstract**

Stability and adaptability of genotypes determine their continuation in farming systems. The traits that contribute stability and adaptation of local landraces of finger millet are not clearly understood. Therefore, seven genotypes of finger millet landraces and one improved variety were studied in Begnas and Kachorwa to determine their adaptiveness and stability considering regression coefficient ( $b_i$ ) and coefficient of determination ( $R^2$ ). The experiment was conducted in farmer's field following farmers' systems of crop management. Genotypes and genotype by environment interactions were highly significant for finger number and grain yield. *Seto Dalle* and *Jhyape* have the highest number of finger in Begnas and Kachorwa respectively. *Syankhole* in Begnas and *Muna* in Kachorwa produced the highest yield. On an average two landraces from Begnas produced higher yield than the improved one. Most landraces performed well in their respective sites. Genotypes in term of finger number and yield exhibited differently for their adaptability and stability. *Arbali Kodo* and *Kalo Jhyape* were stable and adapted to all environments for finger number. *Kalo Jhyape* was stable and adapted to all environments for grain yield. Landraces from Kachorwa were adapted to unfavourable environments. Commonly grown landraces are more stable and adapted to a particular site. Stability and adaptability of genotypes was directly linked to continued cultivation by farmers. Landraces adapted to favourable and unfavourable environments could be important for site-specific variety development.

**Key words:** Adaptability, finger millet landraces, on farm trial, regression, stability

**Introduction**

Finger millet (*Eleusine coracana* (L.) Gaertn) is considered an important crop for poor farmers and soil. It is more important in subsistence farming systems and has paramount significance for food security. Understanding of the extent and distribution of diversity is prerequisite to crop improvement, implementation of conservation programmes and for enhancing and supporting a framework of knowledge on farmer decision-making processes that influence *in situ* conservation. Stability over the years is considered important for site-specific variety development. The adaptability of finger millet could help to make germplasm exchange more efficiently between two ecosites. Therefore we have assessed the adaptability and stability performance of finger millet landraces with respect to improved cultivar.

Evaluation of genotypes for consistent performance in different environments is important in plant breeding programmes. The relative performance of genotypes often changes from one environment to another. The occurrence of a large genotype  $\times$  environment ( $G \times E$ ) interaction poses a major problem for relating phenotypic performance to genetic constitution and makes it difficult to decide which genotypes should be selected. It is important to understand the nature of  $G \times E$  interaction to make testing and ultimately selection of genotypes more efficient. Measuring  $G \times E$  is important to determine an optimum breeding strategy for releasing genotypes with adequate adaptation to target environments. Bilbro and Ray (1976) mentioned that the use of two parameters (adaptation and stability) in conjunction with yield would be of significant benefit in the evaluation and characterization of genetic materials. Genotypes with a stable performance across changing environments, even with modest yield, are considered more relevant than high-yielding cultivars with

inconsistent performance across unpredictable crop seasons in order to provide food security in fragile environments. The decision to release a genotype is usually made on the basis of whether the genotype performance was satisfactory in comparison with the performance of one or more standard cultivars over crop seasons. Thus a major problem is deciding whether the performance of the genotype was satisfactory. Stability analysis has been used by many researchers (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Bilbro and Ray 1976; Guimaras *et al.* 1998; Joshi *et al.* 2003) to decide whether the performance of the genotype is satisfactory.

Nepal is rich in finger millet genotypes, which is grown upto 3150 m (Upreti 1999). About 790 accessions have been collected from various parts of Nepal (Gupta *et al.* 2000). In addition to this large diversity within *Eleusine coracana*, two wild species—*E. indica* and *E. aegyptica*—are found (Baniya 1999; Joshi and Joshi 2002; Upadhyay and Joshi 2003). Finger millet diversity at household level has been most positively influenced by the number of *bari* land parcels (Rana *et al.* 2000c; Joshi and Joshi 2002). However, for various reasons the diversity in finger millet is decreasing rapidly. High variation in finger millet was reported in Kaski landraces by Tiwari *et al.* (2003) who described farmers' descriptors of landraces including *Arbali Kodo*, *Kalo Jhyape*, *Kukurkane*, *Seto Dalle*, *Syankhole* and *Okhale-1*. *Kalo Kodo* is being cultivated by few farmers in limited areas (Tiwari *et al.* 2003). A total of 12 different farmer-named landraces were reported and *Rato Kodo* was the most dominant in Jumla (Rana *et al.* 2000b). In Bara, Muna and *Jhalari* were the common landraces among 6 different farmer-named landraces (Rana *et al.* 2000a). Rana *et al.* (2000c) reported 24 different farmer-named landraces in Kaski. *Jhyape* was the most common landraces followed by *Dalle* and *Dudhe Kodo*. Rana *et al.* (2000c) reported that the stability of yield over years was one of the major considerations for growing a particular landrace. Therefore this study has the objective of assessing adaptability and stability of finger millet landraces.

### **Materials and methods**

Eight genotypes consisting of 2 landraces from Kachorwa, 5 landraces from Begnas and one improved variety were studied for their adaptability and stability (Table 1). The most commonly grown landraces in Kachorwa and Begnas were selected. On-farm experiments were conducted in two sites, Begnas (altitude 848 m, longitude 84°09' E, latitude 28°11'N with *Rato Chimte* soil and Kachorwa (altitude 85 m, longitude 85°10'07"E, latitude 26°53' N with sandy loam soil) in the 2003 growing season. Local landraces were collected from farmers whereas *Okhle-1*, which was released in 1980 (NARC 2000), was collected from HCRP, NARC and used as standard check.

Test entries were laid out in a randomized complete block design in 3 replications. Plot size was 0.5 × 1 m in Begnas and 0.9 × 1 m in Kachorwa. A nursery was raised for which seeds were seeded on 21 June in Begnas and 26 June in Kachorwa. Because of poor germination of landraces from Kachorwa in Begnas, these were reseeded on 17 July. In Begnas, 46- and 21-day-old seedlings and in Kachorwa 21-day-old seedlings were transplanted at 15 × 10 cm spacing. Seedling raising, transplanting, manuring, weeding and other agronomic practices were the same as farmers' management. Farmers and researchers frequently monitored the trials. The population structure of these landraces from each experiment site is presented by Amgain *et al.* and Tiwari *et al.* in this volume (see pp. 84 and 96, respectively). Climatic parameters were also recorded at each site and presented graphically. For Kachorwa, climatic data for the finger millet growing season were collected from the meteorological station at Simara and, for Begnas, these were collected from the Fish Research Station, Begnas.

**Table 1. Finger millet landraces studied for their adaptability and stability.**

Genotype	Source	Remarks
Arbali Kodo	Begnas, Kaski	Landrace
Kalo Jhyape	Begnas, Kaski	Landrace
Kukurkane	Begnas, Kaski	Landrace
Seto Dalle	Begnas, Kaski	Landrace
Syangkhole	Begnas, Kaski	Landrace
Muna	Kachorwa, Bara	Landrace
Jhalari	Kachorwa, Bara	Landrace
Okhale-1	HCRP, Kavre	Improved (standard check)

Although many characters of these genotypes were examined, only the finger number and grain yield data were analyzed and reported. These traits are important for evaluating genotype adaptability and stability and farmers also considered these traits in evaluating landraces. Five plants were tagged to count finger number/ear and yield was measured from the whole plot. These traits were measured as described in IBPGR (1985).

Analysis of variance (ANOVA) was conducted according to procedures outlined by Gomez and Gomez (1984). The environment-wise ANOVA indicated the genotypic effects were significant at both sites. After testing the homogeneity of variances, data were subjected to stability analysis. Stability analysis was carried out following the model of Finley and Wilkinson (1963) and as described by Bilbro and Ray (1976). In brief, the arithmetic average of their respective traits for a given location was considered as site mean. We had only two sites and blocking was effective; therefore block means was used as site mean. Regression analyses were used to ascertain the stability and adaptation of the genotypes. The site mean was used as the independent variable and the individual cultivar yield was used as the dependent variable. Given the results of regression coefficients ( $b$ ) and coefficient of determination ( $R^2$ ), adaptation and stability of each genotype were interpreted respectively. If  $b$  was not significantly different from 1.0, the genotype was considered adapted to all environments. If  $b$  was significantly larger than 1.0, the cultivar was considered better adapted to favourable environments. If  $b$  was significantly smaller than 1.0, the cultivar was considered better adapted to unfavourable environments. A genotype was considered stable unless its  $R^2$  value was significantly smaller than that of the standard genotype (Bilbro and Ray 1976). The square root of the  $R^2$  was tested to see if it differed significantly from that of the standard variety. Statistical procedures were followed as described by Steel and Torrie (1980). MINITAB, MSTAT and MS Excel were used to process and analyze the data.

### Results and discussion

Analysis of variance over locations indicated high significance for finger number and grain yield (Table 2). Locations were significantly different for these two traits. Genotypes and genotype  $\times$  location interactions were highly significant for these traits. These two sites represent mid-hill and lowland, which are agroecologically different even though climatic parameters were not greatly different between these two sites (Figure 1). Soil, altitude and genotypes may be the major factors for observing significant variations. These significant sources of variation indicated the property of landraces adapted to specific sites and existence of developing site-specific stable genotypes.

Grain yield and finger number are given in Table 3. Finger number/ear was highest in *Seto Dalle* followed by *Kukurkane* in Begnas and *Jhalari* followed by *Muna* in Kachorwa. On an average, *Seto Dalle* produced the highest finger number. *Jhalari* produced the lowest finger number in Begnas and *Syangkhole* produced the lowest in Kachorwa. Most of the landraces did well in their respective site where farmers are cultivating. Five landraces had higher number of fingers than *Okhale-1*, which is the released variety recommended for mid- and

foothills in Nepal. In Begnas, all landraces collected from Begnas produced higher number of fingers than *Okhale-1*. Similarly, landraces collected from Kachorwa produced higher number of fingers than *Okhale-1*. *Syankhole* in Begnas and *Muna* in Kachorwa produced the highest grain yield. On average *Syankhole* produced highest grain yield. This was because these landraces were not adapted to cross-sites. The lowest grain yielders were *Muna* and *Seto Dalle* in Begnas and Kachorwa respectively. Two and four landraces produced more grains than *Okhale-1* in Begnas and Kachorwa respectively. For site-specific varietal works, *Muna* and *Jhalari* for Kachorwa, *Syankhole* and *Seto Dalle* for Begnas can be selected. These variations for finger number and grain yield can be potential sources for participatory crop improvement. Rana *et al.* (2000c) reported much variability in yield level for different landraces within across-wealth categories. Production was the major determining factor for area coverage. *Jhyape* was the most dominant landrace cultivated for its early maturity, higher yield, good taste, easy harvesting (Rana *et al.* 2000c). *Seto Dalle* is preferred for its white grains as compared to brown. *Samdhi Kodo*, a white grain finger millet, is highly preferred. Both these traits—finger number and grain yield—reflect the ear size which is a main selection criteria of farmers (Baniya *et al.* 2003). Study of farmer's selection criteria is essential in finger millet because the majority of farmers save seed themselves (Baniya *et al.* 2003).

**Table 2. Analysis of variance over locations for finger number and grain yield.**

Group	Source	df	Finger number		Grain yield	
			MS	P	MS	P
1	Locations	1	1.900	0.329	3649581.61	0.026
2	Replication/location	4	1.544		306311.85	
3	Genotypes	7	2.885	0.000	2145591.53	0.000
4	Location x genotype	7	5.965	0.000	5989739.72	0.000
5	Error		0.474		330164.15	
	CV (%)		11.98		22.29	
	SE of means (group 1)		0.25		112.97	
	N		24		24	
	SE of means (group 3)		0.28		234.57	
	N		6		6	
	SE of means (group 4)		0.39		331.74	
	N		3		3	

**Table 3. Grain yield and finger number of finger millet landraces over locations in 2003.**

Genotype	Finger/ear, no.			Yield, kg/ha		
	Begnas	Kachorwa	Average	Begnas	Kachorwa	Average
Arbali Kodo	6.53	6.08	6.31	3286.67	1092.59	2189.60
Kalo Jhyape	5.80	6.33	6.07	3126.67	2314.82	2720.74
Kukurkane	6.87	5.67	6.27	3280.00	2629.63	2954.82
Seto Dalle	7.13	6.33	6.73	3406.67	981.48	2194.07
Syankhole	5.73	4.42	5.06	4813.33	2462.96	3638.15
Okhale-1	5.20	4.92	5.06	3313.33	2388.89	2851.11
Muna	4.47	6.75	5.61	1133.33	3655.56	2394.44
Jhalari	2.67	7.08	4.88	466.67	2888.89	1677.78

Stability and adaptability parameters for grain yield are shown in Table 3. All genotypes are labeled as A, H or L, which means adapted to all, favourable (high-yielding) and unfavourable (low-yielding) environments respectively for finger number and grain yield.

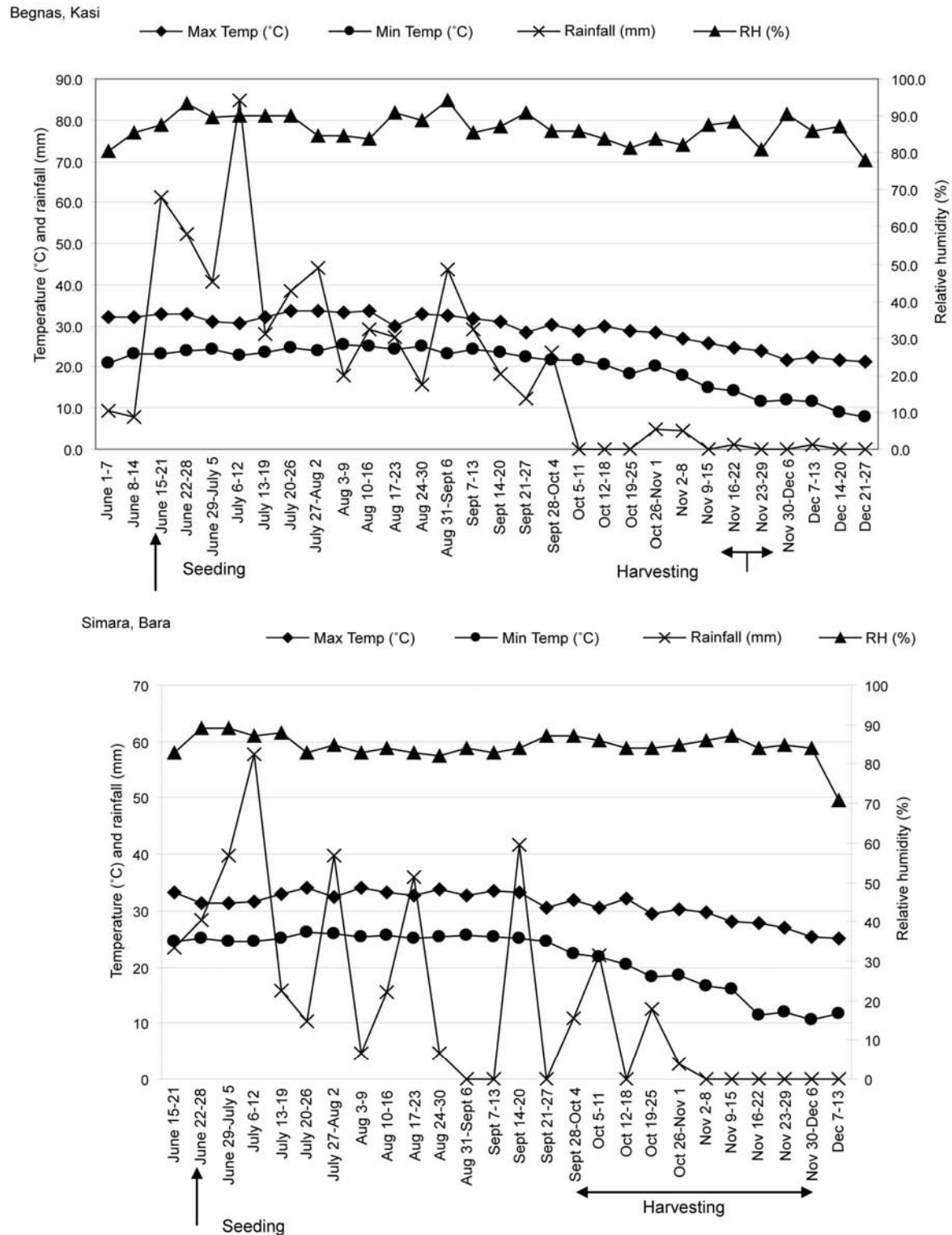


Figure 1. Climatic parameters of Begnas and Kachorwa sites during finger millet growing seasons 2003.

Wide variation was found in regression coefficients and coefficients of determination. Regression coefficients range from  $-1.236$  to  $3.76$  for finger number and  $-2.876$  to  $3.624$  for grain yield. Similarly  $r^2$  ranges from  $3.1$  to  $79.6$  for finger number and  $35.8$  to  $85.7$  for grain yield. The  $r^2$  of some genotypes was very small. This is possibly because of testing genotypes in few environments. Saeed *et al.* (1984) reported that testing genotypes at more locations is more important rather than testing in more years. But, testing genotypes over years will be more important for securing high yield along with conservation of genetic resources in a particular site.

### Finger number

Four genotypes for finger number had a  $b_i$  value significantly different from 1 and four genotypes had  $r^2$  value significantly smaller than that of the standard check, *Okhale-1*. Four varieties were adapted to all environments. *Muna* and *Jhalari*, landraces from Kachorwa were adapted to favourable environments. *Syankhole* and *Kukurkane* were adapted to unfavourable environments for finger number.

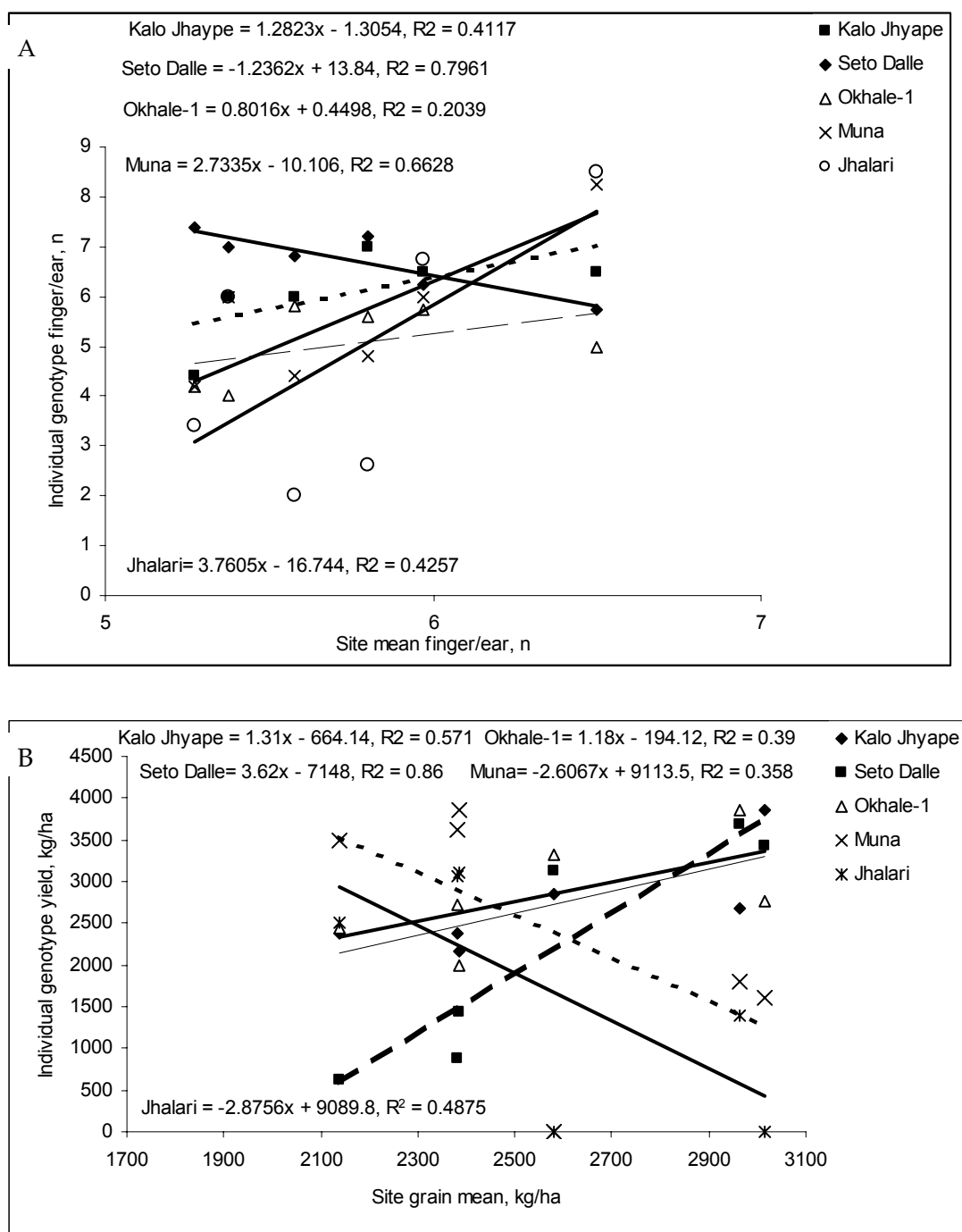
Regression lines of five varieties (*Kalo Jhyape*, *Seto Dalle*, *Muna*, *Jhalari* and *Okhale-1*) are given in Figure 2. *Kalo Jhyape* was stable and adapted to all environments. *Seto Dalle* was unstable but adapted to all environments. *Muna* was unstable but adapted to favourable environments. The stable variety *Jhalari* was adapted to a favourable environment. The plot of these varieties in Figure 3 clearly shows that varieties in the lower left quadrant respond less to change in environment. Varieties of the upper right quadrant had above-average finger number and regression coefficients greater than 1. Therefore, they are good for favourable environments.

### Grain yield

Varieties with  $b_i$  value significantly different from 1 were *Arbali Kodo*, *Seto Dalle*, *Syankhole*, *Muna* and *Jhalari* for grain yield. None of the varieties had  $r^2$  value significantly smaller than that of *Okhale-1*. Only *Muna* had a smaller  $r^2$  value than *Okhale-1* but not significant. Most of the landraces were stable. Three varieties (*Kalo Jhyape*, *Kukurkane* and *Okhale-1*) are defined as ideal genotypes because they are adapted to all environments, are stable and above average in yield ability. Three varieties were adapted to favourable environments, and two landraces *Muna* and *Jhalari* were adapted to unfavourable environment. Variability in stability among these varieties was not observed but high variability was noticed for adaptability. From these varieties, site-specific as well as wider adaptability varieties can be developed. For wide stability, landraces *Kalo Jhyape* and *Kukurkane* can be used.

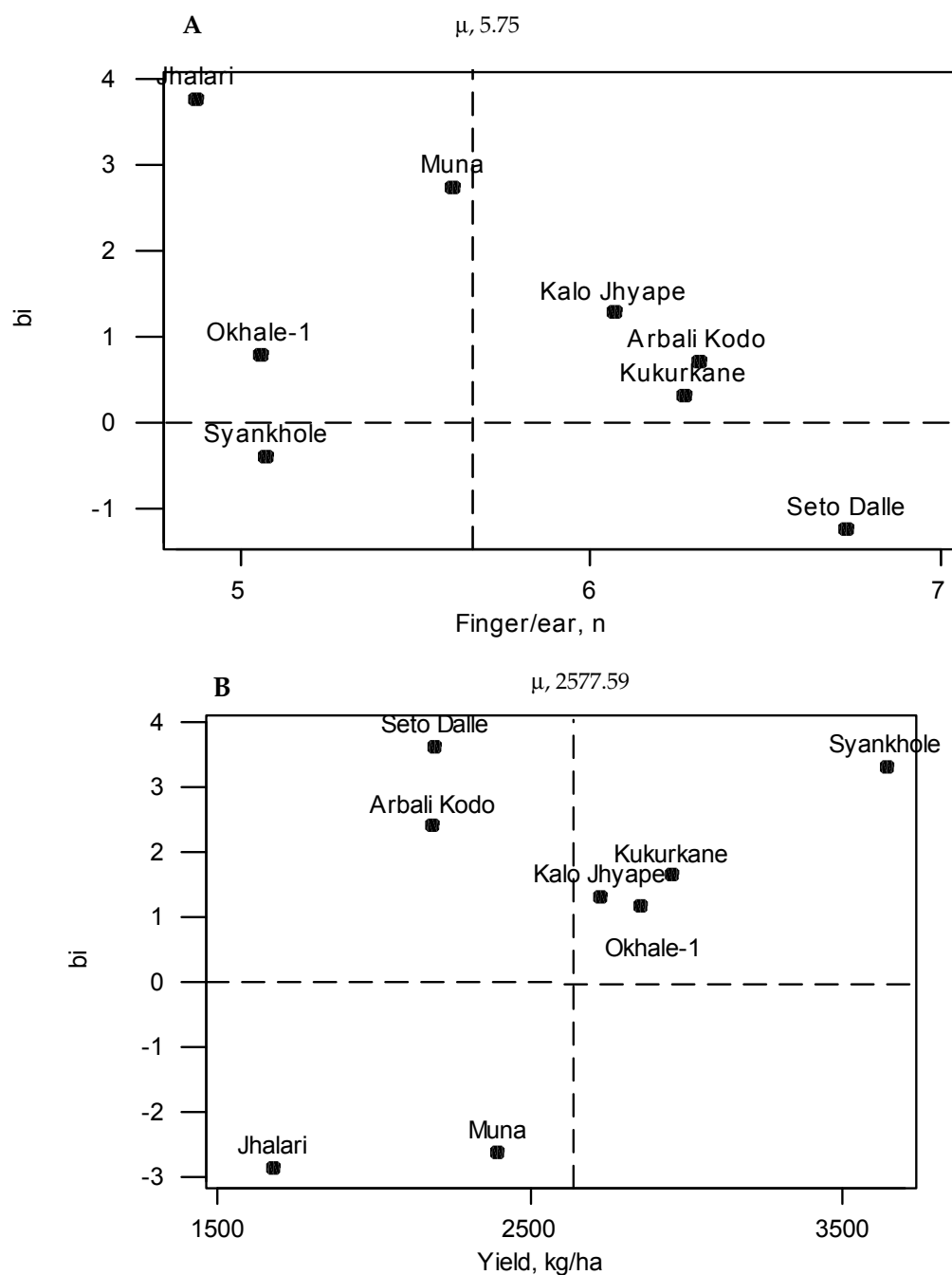
As expected from stability analysis, Figure 2 shows that  $G \times E$  interactions were very high. Individual grain yields vs. site mean yield of 5 varieties are shown in Figure 2. *Seto Dalle* had the highest regression coefficient. *Muna* and *Jhalari* had negative regression coefficient. *Seto Dalle* was stable and adapted to favourable environments.

A scatter plot of all these genotypes considering regression coefficient and genotype mean yield is shown in Figure 3. This plot helps to select genotypes easily for all low-yielding and high-yielding environments. Genotypes falling in the upper right quadrant have above-average yields and regression coefficients greater than 1. These genotypes, e.g. *Syankhole*, are highly responsive to the environments, i.e. they are good for high-yielding environments. Genotypes (*Jhalari* and *Muna*) in the lower left quadrant respond less to changes in environment, as their regression coefficients are less than 1 with yield below average. They are less adapted to favourable environments and differ in adaptation to unfavourable environments. Farmers can grow genotypes falling in the upper right quadrant if the environment is favourable and can select genotypes in the lower left quadrant if the environment is poor. Genotypes in the lower left quadrant can contribute as parents in finger millet improvement for stressed conditions. Varieties such as *Okhale-1*, *Kalo Jhyape* and *Kukurkane* have regression coefficients near 1 and mean yields above average and are considered to be stable.



**Figure 2. Relationship between individual genotype and site mean for finger number (A) and grain yield (B) of finger millet landraces.**





**Figure 3. Placement of finger millet landraces in the ground of regression coefficient vs genotype mean for finger number (A) and grain yield (B).**

### Stability and adaptability

Stability and adaptability of finger millet is considered higher than in other crops. Most of the varieties studied here were stable and adapted to diverse environments. Upreti (1999) reported that landraces cultivated in mid-hills are very poorly suited to high altitude. Upreti (1999) also suggested that landraces should be characterized and evaluated under the same environment as collection sites for better utilization. Because of its hardness in nature in terms of insect pests and drought tolerance, finger millet is cultivated without fertilizer in areas where other cereals cannot be grown (Subedi 1991; Rana *et al.* 2000c; Joshi and Joshi 2002). The genotypes with traits along with stability over years and adaptability to marginalized lands would be ideal for increasing production and productivity. Stability and adaptability of these varieties differed with studied traits. For example *Kukurkane* was unstable and adapted to unfavourable environments for finger number but it was stable and adapted to all environments for grain yield (Table 4). Finger millet is grown for grain yield so this trait should be given prime importance for this type of study. After the multiplication trials over years *Okhale-1* was released for general cultivation. However most of the landraces are better than *Okhale-1* in terms of stability and adaptability. The most common landrace, *Kalo Jhyape* in Kaski (Rana *et al.* 2000c), was stable and adapted to all environments. Similarly the common landraces in Bara, *Muna* and *Hilary* (Rana *et al.* 2000a), were stable but adapted to unfavourable environments.

**Table 4. Stability and adaptability parameters of finger millet landraces.**

Genotype	Finger/ear <sup>†</sup>			Grain yield		
	bi	R <sup>2</sup>	Adaptation	bi	R <sup>2</sup>	Adaptation
Arbali Kodo	0.717	24.4	A	2.40C	38.6	H
Kalo Jhyape	1.282	41.2	A	1.313	57.1	A
Kukurkane	0.334C	3.1D	L	1.64	59.4D	A
Seto Dalle	-1.236	79.6D	A	3.624C	85.7D	H
Syankhole	-0.393C	4.8D	L	3.323C	75.1D	H
Okhale-1	0.802	20.4	A	1.181	39.5	A
Muna	2.733C	66.3D	H	-2.607C	35.8	L
Jhalari	3.76C	42.6	H	-2.876C	48.8	L

<sup>†</sup> b, Regression coefficient.  $r^2$ , coefficient of determination. A, H, L, adapted to all, favourable and unfavourable environments, respectively. C, Significantly different from 1. D, Significantly smaller than that of the standard genotype.

### Conclusion

Landraces are better adapted and stable to their sites where these are cultivated, even though variation exists among these landraces in terms of adaptability and stability. Studies on adaptation and stability should be focused on the most important traits because genotypes differ in their stability and adaptability with respect to traits. Most of the common landraces are stable but variation exists within common landraces for their adaptability. Farmers were able to select stable genotypes adapted to diverse environments. This type of study should be conducted over many years and locations to generalize the conclusions.

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## Intra- and interpopulation variation in finger millet (*Eleusine coracana* (L.) Gaertn) landraces grown in Kachorwa, Bara, Nepal

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### Abstract

Finger millet [*Eleusine coracana* (L.) Gaertn] is an important food crop for poor and hilly people of Nepal. The many ecogeographical niches of the country have resulted in large diversity in finger millet landraces. This study was carried out from June to December 2003 at the *in situ* site of Kachorwa VDC, Bara, Nepal to assess the inter- and intrapopulation variability and relationship between different traits in finger millet landrace populations. Five landraces from mid-hill (Begnash, Kaski), and two landraces from central Terai (Kachorwa, Bara) of Nepal were collected, with one improved Nepalese cultivar *Okhale-1*. Thirty plants from each population were studied. All the 18 studied quantitative traits showed large intrapopulation variability in all populations. Eight quantitative traits were more variable in the *Seto Dalle* population, indicating the operation of natural forces of evolution on its population. The shortest duration of flowering and maturity with high variability was observed in *Jhalari*. A total of 28 morphological classes for 11 qualitative characters were identified in finger millet populations with higher polymorphism in ear shape (5 classes) followed by grain colour (4 classes). Spikelet shattering was found only in *Jhalari* and *Muna*. The *Kalo Jhyape* population showed non-synchronous ear maturity with possibility of its fixation. Quantitative traits except tiller number ( $\beta_1=1.5$ ), finger length ( $\beta_1=1.277$ ), finger width ( $\beta_1=-0.061$ ), and grain yield/plant ( $\beta_1=1.277$ ) were normally distributed without significant skewness. Grain yield/plant was negatively correlated with days to flowering ( $r=-0.397$ ), and days to maturity ( $r=-0.351$ ). However it was positively correlated with tillers/plant ( $r=0.526$ ), leaf number ( $r=0.514$ ), blade length of flag leaf ( $r=0.330$ ), blade breadth of flag leaf ( $r=0.401$ ), finger width ( $r=0.565$ ), grain/spikelet ( $r=0.591$ ), and finger number ( $r=0.482$ ). Terai and hilly landraces were grouped in two different groups. The inter- and intrapopulation variation found in these landraces could be useful for finger millet improvement. The landraces of central Terai could serve as the genepool for earliness and yield purposes for Terai condition.

**Key words:** Correlation, *Eleusine coracana*, fixation, polymorphism, population structure

### Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is an important crop in the hills of Nepal. It is grown in dry and extremely marginal areas in the plains, relayed with maize in the majority of mid-hill areas, and grown as a monocrop or in a mixed cropping system with cereals and/or legumes in the high hills and in some western hills of Nepal (Baniya *et al.* 2003a). In Terai, it is a summer as well as a rainy season crop.

However, finger millet is the livelihood of hill farmers of Nepal. Nepalese hill farmers use it as their main diet, as tiffin, for making *Jand* (local fermented and non-distilled alcoholic drink), *Rakshi* and *Tumba* (local fermented and distilled alcoholic drink), and sometimes for animal feed. Finger millet straw is also good animal forage in the mid-hills.

In central Terai it is used as tiffin, for making *haluwa*, *roti* and *chokha*. It is also assumed to be a good diet for pregnant women and for treatment of animal diarrhea (Rana *et al.* 2000). People in the Janakpur – Jaleshwar region use it during fasting on *Jeetiya* (a festival of women). Thus it has strong social and cultural ties in the Nepalese farm community.

Finger millet has high nutritional value especially for calcium, iron, methionine and manganese (ICRISAT 2004). Its slow digestion indicates low blood sugar levels after a finger millet diet thereby it is a safer food for diabetics (LMA 2004). In Nepal, there are many

opportunities for its promotion as income-generating enterprise or as a raw material for agrobased industries (Sthapit *et al.* 1993). Except for promotion of it for some bakery products in Pokhara valley (Bhandari *et al.* 2005), finger millet only occupies the traditional consumption pattern and market channel.

It is an allotetraploid with 36 chromosomes and a haploid genome size of about 2700 Mb (Bennett and Leitch 1995). It is productive in a wide range of environments and growing conditions, from southern Karnataka state in India to the foothills of the Himalayas in Nepal, and throughout the middle-elevation areas of Eastern and Southern Africa (ICRISAT 2004). Thus, finger millet diversity in Nepal is rich at both varietal and population levels (Baniya *et al.* 1992), and this diversity could be used for variety improvement. However, meager work has been conducted to characterize and utilize this diversity. Thus, this study was conducted to assess inter- and intrapopulation variability and relation between different traits of finger millet landraces in central Terai conditions, Nepal.

### **Material and methods**

The upland field with sandy loam soil at 85 masl of Kachorwa VDC, Bara (26°53'N, 85°10'07"E) was selected. Five landraces (*Syankhole*, *Arbali Kodo*, *Seto Dalle*, *Kalo Jhyape*) and *Kukurkane* from Begnas, Kaski, two landraces (*Muna* and *Jhalari*) from Kachorwa, Bara, and one improved cultivar (*Okhale-1*) were used for this study. Landraces were selected on the basis of their popularity and distribution within ecosites. Around 150 seeds of each landrace were collected from farm households and sown at the end of June 2003. The 25-day-old seedlings were transplanted to 0.9 m<sup>2</sup> plots with spacing of 15 × 10 cm. Farmers' cultural practices were used. Thirty individual plants from each entry were tagged and their agronomic and morphological characters studied according to descriptors for finger millet (IBPGR 1985). Intra- and interpopulation diversity was calculated for quantitative and qualitative traits. All populations were pooled for correlation study. The summary of the weather report of the evaluation site can be found in Joshi *et al.* (see p. 73). Data were analyzed using the statistical program Minitab and SPSS.

### **Results and discussion**

#### **Intrapopulation structure**

##### **Quantitative traits**

The higher mean and range for tiller number, blade width of flag leaf, and finger number is found in *Muna*, for grains/spikelet in *Jhalari*, and for plant height in *Kalo Jhyape* (Table 1). Mean plant height was shorter in *Jhalari* and *Muna*, which were also reported by Tiwari *et al.* (2003) using the same varieties in Nepalese hill conditions. Thus, these populations can serve as the best genepool for increment of the respective characters.

Higher mean with higher coefficient of variation (CV) was found in *Okhale-1* for finger length. Higher CV with high standard error was also found in *Muna* for ear exertion and finger width, in *Jhalari* for blade length of flag leaf and for finger number, in *Seto Dalle* for plant height, total leaf number, leaf sheath length, leaf sheath width, leaf blade width, blade width of flag leaf, grains/spikelet and grain yield/plant, in *Kalo Jhyape* for leaf blade length, and in *Kukurkane* for tiller number (Table 1). Since landraces showing high variability were useful in breeding programmes (Baniya *et al.* 2003b), these landrace populations are the best genepool for respective characters. Similarly, shorter duration for flowering and maturity with higher variability for these characters was found in *Jhalari* followed by *Muna* which could be used as the source genepool for developing a short-duration variety.

Table 1. Mean, coefficient of variation and range of different traits influencing outcrossing among rice landraces, Kachorwa, Bara, Nepal.

Character	Muna	Jhalari	Syank-hole	Arbali Kodo	Seto Dalle	Kalo Jhyape	KukurKane	Okhale-1
Plant height, cm	Mean±SE	66.50±1.45	86.85±1.78	90.63±2.76	94.73±3.16	103.63±3.02	87.37±1.78	101.73±2.52
	CV %	11.98	10.50	16.88	17.06	15.97	11.21	13.62
	Range	48.00–84.00	71.00–105.00	48.00–114.00	65.00–119.00	53.00–129.00	62.00–103.00	75.00–125.00
Tiller no.	Mean±SE	1.37±.1	1.63±.13	1.10±.07	1.33±.11	1.20±.1	1.47±.13	1.10±.05
	CV %	40.69	42.20	37.09	46.51	45.91	49.79	27.74
	Range	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–3.00	1.00–2.00
Culm thickness, mm	Mean±SE	2.75±.20	3.45±.06	3.73±.15	3.44±.15	3.70±.13	3.68±.13	3.98±.10
	CV %	33.12	10.14	21.96	23.11	19.29	19.66	13.94
	Range	1.50–4.60	2.80–4.20	2.20–5.40	2.20–4.90	2.30–5.00	2.20–5.50	2.80–5.20
Leaf, no.	Mean±SE	11.40±.35	12.31±.52	11.45±.52	10.02±.54	13.79±.61	13.37±.38	11.59±.35
	CV %	17.02	21.65	24.77	27.96	23.92	15.65	16.40
	Range	7.00–15.00	5.00–17.00	4.00–18.00	5.00–17.00	8.00–20.00	8.00–17.00	8.00–15.00
Leaf sheath length, cm	Mean±SE	9.69±.31	11.41±.25	9.22±.32	9.57±.48	11.38±.5	9.73±.36	10.50±.3
	CV %	17.89	11.66	19.14	25.81	23.74	20.58	15.90
	Range	6.00–12.50	8.50–13.50	5.50–14.00	0.40–13.00	6.50–16.00	5.50–14.50	7.00–13.00
Leaf sheath width, cm	Mean±SE	0.50±.01	0.47±.01	0.48±.01	0.47±.02	0.48±.01	0.47±.02	0.50±.01
	CV %	15.20	17.51	17.96	26.80	20.01	23.50	20.00
	Range	0.30–0.70	0.30–0.70	0.30–0.70	0.30–0.80	0.30–0.70	0.30–0.70	0.30–0.80
Leaf blade length, cm	Mean±SE	38.05±1.33	28.20±1.03	31.88±.75	32.65±.96	34.03±1.34	28.67±.81	32.93±.84
	CV %	19.18	19.16	12.73	15.14	21.22	15.52	13.77
	Range	22.00–46.50	16.00–37.50	24.00–40.50	22.00–44.00	14.00–50.00	21.00–38.00	21.00–41.00
Leaf blade width, cm	Mean±SE	1.02±.01	0.93±.02	0.91±.02	0.91±.04	0.91±.02	0.92±.02	0.91±.02
	CV %	6.66	12.25	12.82	24.30	14.78	12.94	12.91
	Range	0.90–1.20	0.70–1.30	0.60–1.10	0.70–1.80	0.60–1.30	0.70–1.20	0.70–1.10
Flag leaf blade length, cm	Mean±SE	22.71±1.03	20.52±.81	26.22±1.04	22.94±1.27	24.41±1.2	19.53±1.02	22.52±1.08
	CV %	24.85	20.69	21.48	28.36	26.65	28.76	25.97
	Range	9.00–32.50	10.50–29.00	15.00–36.00	14–34.50	9.50–39.00	7.50–32.50	11.00–36.50
Flag leaf blade width, cm	Mean±SE	0.81±.02	0.77±.02	0.69±.02	0.74±.02	0.68±.02	0.73±.02	0.72±.02
	CV %	15.86	17.77	16.33	20.65	16.87	16.93	17.17
	Range	0.50–1.00	0.40–0.90	0.50–1.00	0.50–1.00	0.50–0.90	0.50–1.00	0.50–1.00

Table 1. (cont'd.)

Character	Muna	Jhalari	Syank-hole	Arbali Kodo	Seto Dalle	Kalo Jhyape	KukurKane	Okhale-1
Ear exertion, cm	Mean±SE	10.16±.71	8.19±.45	12.89±.72	14.41±.53	12.58±.6	12.02±.55	9.22±.77
	CV %	38.42	29.70	29.79	19.32	26.21	25.21	45.98
	Range	0.50–13.00	1.00–12.00	0.00–17.50	9.50–20.50	1.00–17.50	4.50–17.00	0.00–14.00
Finger length, cm	Mean±SE	5.13±.14	5.66±.13	5.16±.14	4.92±.18	8.45±.29	7.23±.20	9.72±.51
	CV %	14.95	12.96	15.16	19.71	18.81	15.77	28.78
	Range	3.50–7.00	4.00–7.00	3.50–6.50	3.00–7.00	5.00–11.00	5.00–10.50	4.50–15.00
Finger width, cm	Mean±SE	0.76±.03	0.95±.01	0.80±.02	0.65±.02	0.76±.02	0.80±.02	0.74±.02
	CV %	21.94	10.08	18.70	20.17	16.67	19.97	18.56
	Range	0.50–1.00	0.70–1.00	0.60–1.00	0.50–1.00	0.50–1.00	0.50–1.00	0.50–1.00
Finger no.	Mean±SE	6.70±.29	4.00±.17	4.60±.28	5.00±.24	5.17±.23	5.57±.23	4.20±.17
	CV %	22.60	22.57	24.37	25.30	24.27	22.96	22.89
	Range	4.00–10.00	2.00–5.00	3.00–7.00	3.00–7.00	3.00–8.00	3.00–9.00	1.00–6.00
Grains/spikelet, no.	Mean±SE	232.00±12.33	235.71±10.99	210.00±19.62	143.00±11.85	193.28±11.57	172.43±9.53	198.46±11.91
	CV %	27.62	24.67	36.20	42.29	32.24	30.30	31.76
	Range	145.00–380.00	111.00–370.00	71.00–300.00	39.00–246.00	66.00–358.00	87.00–299.00	100.00–320.00
Grain yield/plant, g	Mean±SE	5.13±.51	3.96±.36	2.04±.24	1.32±.22	2.70±.31	3.15±.44	2.43±.23
	CV %	52.24	58.27	46.26	85.33	62.14	76.99	50.26
	Range	1.10–10.96	0.96–8.42	0.72–3.99	0.21–5.01	0.21–6.95	0.67–10.84	0.38–4.62
Days to flowering	Mean±SE	74.87±.62	113.70±.47	119.31±1.16	126.67±.98	114.86±.56	115.70±.39	110.17±.69
	CV %	4.56	2.19	5.25	4.05	2.63	1.89	3.39
	Range	70.00–85.00	110.00–119.00	110.00–145.00	116.00–41.00	111.00–123.00	113.00–121.00	101.00–122.00
Days to maturity	Mean±SE	99.58±.69	152.82±.62	153.67±1.08	158.04±.52	156.21±.36	153.83±.34	143.38±.71
	CV %	3.54	2.17	2.74	1.62	1.27	1.22	2.68
	Range	93.00–107.00	142.00–159.00	144.00–157.00	151.00–62.00	151.00–159.00	149.00–157.00	140.00–151.00



High variability for eight studied quantitative characters was found in the *Seto Dalle* population (Table 1), showing greater possibility to improve this population. Greater mutation, migration and recombination may lead this population towards higher variation. However, low variability was observed in most of these populations in Begnas conditions (see Tiwari *et al.*, p. 96). *Seto Dalle* is a landrace of the Begnas area, which may get stress conditions in *Terai*, causing the expression of many genes.

### Qualitative traits

Variation in population of different qualitative traits was observed in finger millet landraces; spikelet shattering was found in central *Terai* landraces *Muna* (0.333) and *Jhalari* (0.345) only. Tiwari *et al.* (p. 96) reported that these landraces did not show spikelet shattering in Begnas conditions; however, *Okhale-1*, *Kalo Jyape* and *Kukurkane* expressed this character. *Kalo Jhyape* population has non-synchronous ear maturity. Discontinuity of spikelets on finger was observed on *Muna* (0.70), *Jhalari* (0.30), *Arbali Kodo* (0.067), *Seto dalle* (0.462), *Kalo Jhyape* (0.931) and *Okhale-1* (0.862) (Table 2). This showed the possibility of fixation of this gene on *Kalo Jhyape* and *Okhale-1*. However, the population of *Arbali Kodo* shows fixation against this gene. This trait was found absent in Begnas conditions for all varieties. This may be a researchable question: why were the local landraces observed with spikelet shattering? Possibly there is some form of natural adaptation, which is influenced by favourable environmental conditions. The absence of discontinuity of spikelet in Begnas showed that this gene is environmentally influenced and favoured by central *Terai* conditions. Very low proportion of white grain colour (0.036) was also observed in *Okahle-1*, which may be due to the gene flow in recent generations because it was a modern cultivar by origin. The white grain colour was favoured by the farmers of Begnas hill (Rana *et al.* 2000).

### Interpopulation structure

#### Quantitative traits

Maximum interpopulation variation was observed in grain yield/plot (39.27%) followed by finger length (27.30%), ear exertion (25.38%), days to flowering (19.50%), days to maturity (19.24%), grains/spikelet (19.18%), finger number (17.25%), tiller number (16.63%), and plant height (16.12%). Minimum interpopulation variation was observed in leaf sheath width (3.78%) followed by leaf blade width (5.35%), blade width of flag leaf (6.11%), leaf sheath length (8.48%), leaf blade length (9.69%), blade length of flag leaf (6.11%), culm thickness (10.29%), leaf number (10.95%), and finger width (11.18%). Similar results for finger length were found by Tiwari *et al.* (2003). High variation on days to flowering and days to maturity, in contrast with the report of Tiwari *et al.* (2003), may be due to the environmental variation of growing sites.

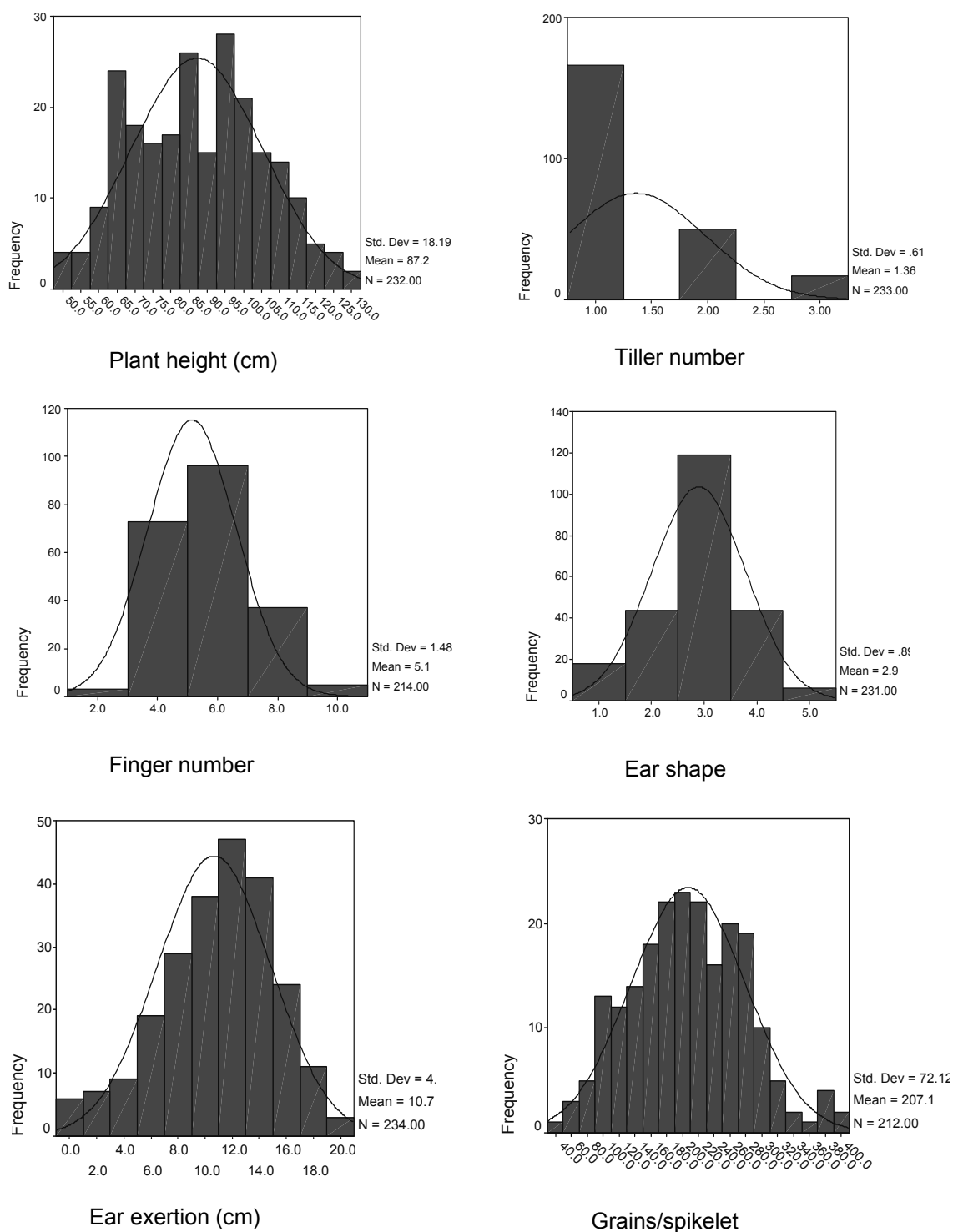
Plant height, ear exertion, finger number/ear and grains/spikelet were normally distributed with little skewness in finger millet population (Figure 1). Similarly, culm thickness, total leaf number, leaf sheath length, leaf sheath breadth, leaf blade length, leaf blade breadth, blade length of flag leaf, blade width of flag leaf, and lodging susceptibility were also normally distributed with little or no skewness.

Tiller number was skewed towards the left ( $\beta_1=1.5$ ). This is due to the high frequency of plants with only one tiller. Finger length was also skewed left ( $\beta_1=1.322$ ), but finger width skewed right ( $\beta_1=-0.061$ ). This shows no good relation between finger length and breadth, and correlation between these two was also non-significant (Table 3). Skewness towards the left ( $\beta_1=1.277$ ) was found in grain yield/plant showing fewer plants with extraordinary yielding capacity (Figure 1).

**Table 2. Proportion of the different characters of finger millet landrace populations in Kachorwa, Bara.**

Traits	Class	Muna	Jhalari	Syan-khole	Arbali kodo	Seto Dalle	Kalo Jhyape	Kukur-kane	Okhale-1
Growth habit	Erect	1	1	1	1	1	1	1	1
Lodging	Low	1	1	1	1	0.962	0.897	1	0.966
susceptibility	Intermediate					0.038	0.103		0.034
Ear shape	Droopy						0.367	0.033	0.207
	Open		0.167	0.071			0.633	0.467	0.138
	Semi-compact	1	0.733	0.571	0.429	0.269		0.500	0.586
	Compact		0.1	0.286	0.536	0.615			0.069
	Fist-like			0.071	0.036	0.115			
Ear size	Small	0.300	0.333	0.321	0.586	0.615	0.167	0.233	0.138
	Intermediate	0.700	0.633	0.643	0.414	0.385	0.567	0.600	0.586
	Large		0.033	0.036			0.267	0.167	0.276
Finger branching	Absent	0.600	0.700	1	1	1	1	1	0.967
	Present	0.400	0.300						0.033
Discontinuity of spikelets on finger	Absent	0.300	0.700	1	0.933	0.538	0.069	1	0.138
	Present	0.700	0.300		0.067	0.462	0.931		0.862
Spikelet shattering	Absent	0.667	0.655	1	1	1	1	1	1
	Present	0.333	0.345						
Grain covering	Exposed				0.333	0.5	1	0.067	0.033
	Intermediate	1	1	1	0.667	0.5		0.933	0.967
Grain colour	White			1					0.036
	Light brown	0.037	0.862		0.800			0.067	0.214
	Copper-brown	0.889	0.138		0.200	0.846	1	0.933	0.750
	Purple-brown	0.074				0.154			
Synchrony of ear maturity	Not synchronous	0.880	0.655	0.607	0.867	0.958	1	0.933	0.207
	Synchronous	0.120	0.345	0.393	0.133	0.042		0.067	0.793
Grain surface	Smooth	1	1	1		0.885	1	0.933	0.828
	Wrinkled				1	0.115		0.067	0.172

For days to flowering (DTF), and days to maturity (DTM), the distribution becomes somewhat platykurtic ( $\beta_{1DTF}=-0.876$ ,  $\beta_{2DTF}=-0.698$ ;  $\beta_{1DTM}=-1.012$ ,  $\beta_{2DTM}=-0.785$ ) with two distinct groups for days to flowering and days to maturity. Since *Muna* and *Jhalari* are *Terai* landraces with significant short duration of cropping and with no photosensitivity and others have long-duration cropping, these two groups are possible. Cluster analysis using all studied traits also showed *Muna* and *Jhalari* as distinct from others. Among hill genotypes, *Kalo Jhyape* was found distinct from the others (Figure 2). The same result was observed by Tiwari *et al.* while using the same varieties and same characters for study in Begnas hill conditions of Nepal.



**Figure 1. Distribution of different traits of finger millet landraces population.**

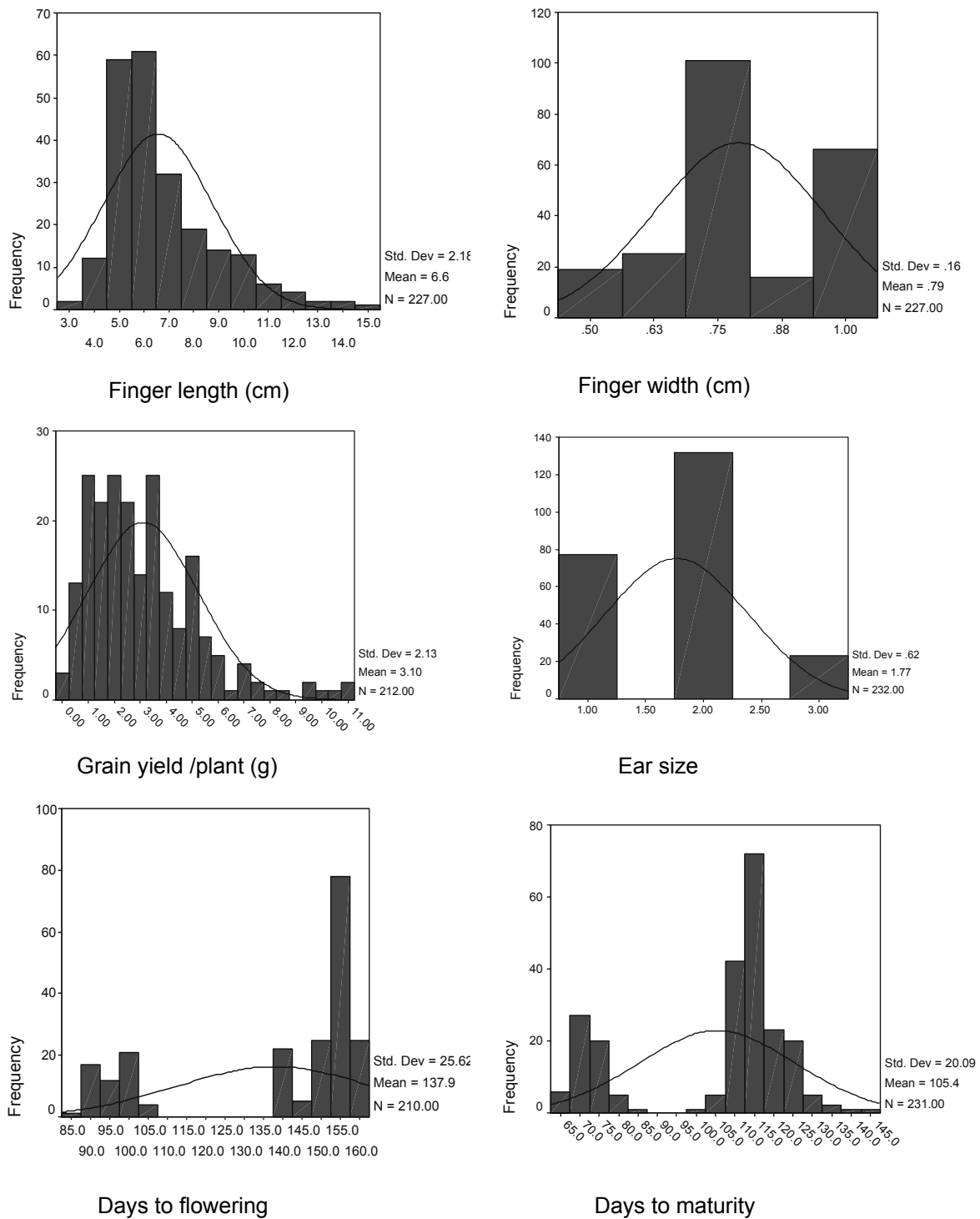
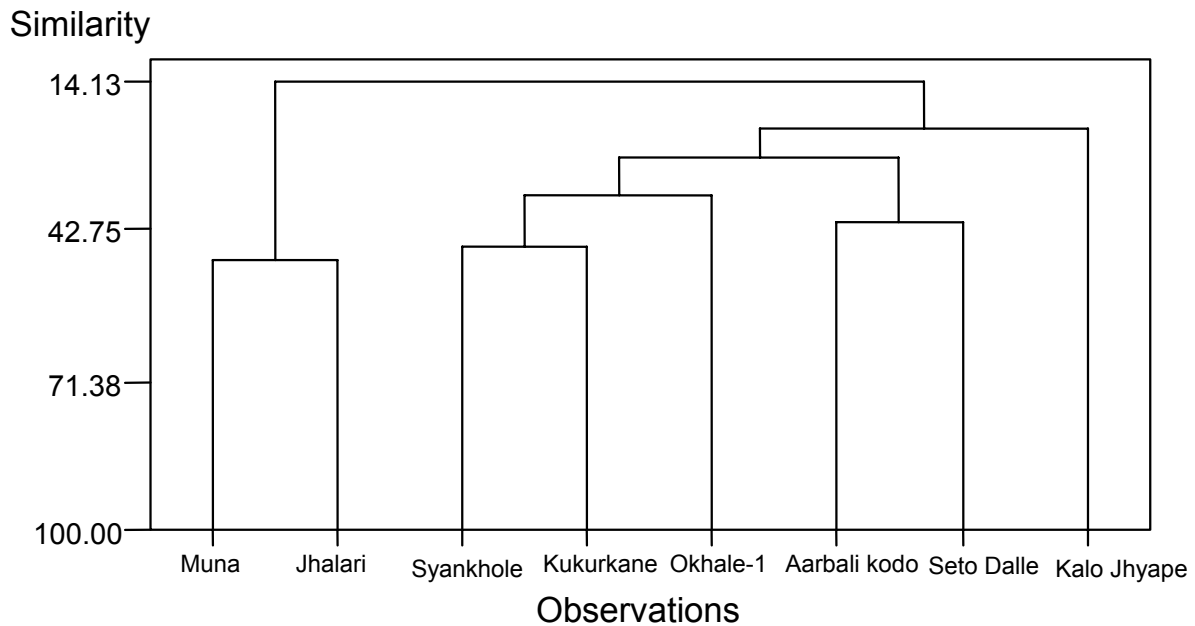


Figure 1 (cont.). Distribution of different traits of finger millet landraces population.

Table 3. Correlation between different traits observed on finger millet landraces.

	Tiller number	Days to flowering	Leaf number	Blade length of flag leaf	Blade width of flag leaf	Finger length	Finger width	Grain / spikelet	Finger number	Grain yield/plot	Ear exertion	Days to maturity
Plant height	-0.108	0.596**	0.277**	0.197**	0.001	0.515**	0.136*	0.020	-0.086	-0.016	0.447**	0.629**
Tiller number		-0.164**	0.063	0.048)	0.066	-0.092	0.167**	0.075	0.184**	0.526**	-0.048	-0.148*
Days to flowering			-0.130*	-0.152	-0.291**	0.150*	-0.137*	-0.422**	-0.371**	-0.397**	0.4**	0.968**
Leaf number				0.150*	0.368**	0.325**	0.325**	0.361**	0.432**	0.514**	-0.122	-0.031
Blade length of flag leaf					0.491**	0.082	0.249**	0.425**	0.249**	0.330**	0.222**	-0.163*
Blade width of flag leaf						0.06	0.157*	0.324**	0.479**	0.401**	0.002	-0.247**
Finger length							-0.015	0.089	-0.005	0.065	-0.006	0.216**
Finger width								0.608*	0.031	0.565**	0.095	-0.108
Grains/spikelet									0.210**	0.591**	0.008	-0.404**
Finger number										0.482**	0.003	-0.387**
Grain yield/plot											-0.114	-0.351**
Ear exertion												0.325**

Note: \*0.05&lt;p&lt;0.01, \*\*p&lt;0.01, n = 210



**Figure 2. Clustering patterns of 8 finger millet varieties grown at Kachorwa, Bara, Nepal.**

### Qualitative traits

We identified 28 morphological classes for 11 qualitative characters on finger millet populations. Among them, 18, 20, 17, 18, 20, 16, 19, and 24 classes were identified from *Muna*, *Jhalari*, *Syankhole*, *Arbali kodo*, *Seto Dalle*, *Kalo Jhyape*, *Kukurkane* and *Okhale-1* populations, respectively (Table 2). Higher polymorphism could be seen in ear shape (5 classes) followed by grain colour (4 classes). However, all populations were erect for growth habit. Ear size was also little skewed towards the left ( $\beta_1=0.187$ ) showing lower frequencies of the large size ear on finger millet population (Figure 1).

### Correlation among different traits

Correlation between 13 qualitative traits showed different degrees of relationship (Table 3). Tiller number, total leaf number, blade length of flag leaf, blade width of flag leaf, finger width, grain/spikelet, finger number/ear was positively correlated ( $P<0.00$ ) with the grain yield/plant. Negative correlation was found for days to flowering ( $-0.397$ ,  $P<0.00$ ) and days to maturity ( $-0.351$ ,  $P<0.00$ ) with grain yield/plant. This may be due to sterility problem and non-adaptability of the hill genotypes having longer days to flowering and days to maturity in central *Terai* condition. Negative correlation of plant height ( $-0.016$ ,  $P<0.819$ ) and ear exertion ( $-0.114$ ,  $P<0.097$ ) with grain yield/plant was also observed.

Farmers use traits like big ear size, well-matured ear, big grain size, free from finger millet blast and insect pest, and non-lodging plants as selection criteria and want new high-yielding varieties with the features of *Dalle* and *Jhyape* local varieties (Baniya *et al.* 2003a). Moreover, as optimum expression of the characters having positive association with yield is important for a breeding programme (Rasmusson and Gengenbach 1980), the relationships observed in our study could be used to develop the varieties asked for by farmers.

## Conclusion

Since intrapopulation variation was observed in different finger millet landraces at central Terai conditions, this variation could be effectively used for the improvement of particular populations in the desired direction. The *Seto Dalle* population, which showed maximum variation in many characters, should be best utilized for the population genetic study, and germplasm conservation and utilization programmes. The local landraces, which showed their best performances in local conditions, could be further improved by better selection procedures from its diverse population. Earliness from local landrace populations could be used in the national finger millet breeding programme.

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## Intra- and interpopulation variation of finger millet (*Eleusine coracana* (L.) Gaertn.) landraces grown at Begnas, Kaski, Nepal

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### Abstract

Finger millet is the third most prioritized crop with respect to food security and nutrition for people in the mountains of Nepal. Owing to the topographical and agroecological variability in the region, farmers in this region grow many landraces to meet their domestic needs. These landraces have been under cultivation for a long time and have been selected on the basis of their popularity, traditional preferences and distribution within the region. The present study on population structure was performed to describe the structure of genetic diversity within and between the finger millet landrace populations. *Muna* and *Jhalari* did not perform well. A considerable level of intra- and interpopulation variability was observed for most quantitative and qualitative traits like plant height, leaf number, length of flag leaf, finger branching, finger type and size, and spikelet shattering. Large -ized ears were encountered in *Seto dalle* and *Syangkhole*. These variations would be useful in a varietal improvement programme. The cluster and principal component analyses grouped the landraces ecogeographically into two distinct groups. This information could be useful for varietal improvements using landraces for wider adaptation.

**Key words:** Genetic diversity, landraces, population, finger millet, *Eleusine coracana*

### Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is commonly known as *Kodo*. People of Begnas place it third in priority crops for food security, after rice and maize (Rijal *et al.* 1997). It is grown as a mono crop, as a relayed crop with maize or in mixed cropping with other cereals and/or legumes in the hills. In *terai*, it is also grown mainly in dry and extremely marginal areas.

Mountains are usually characterized as inaccessible, fragile, diversified and marginal areas. The importance of this crop in the mountains might be due to its hardy nature since it can withstand environmental stresses such as nutrient deficiency, drought, excessive rain, diseases and pests and it has unique features with respect to yield stability and storability. This crop utilizes the residual nutrients and moisture in soil. It is used as an alternative crop in the rice field under rain-fed conditions when the onset of monsoons is delayed or water is not sufficient for transplanting rice (Shrivastava *et al.* 2002). Furthermore, the importance of the crop increases with the increase in altitude because the cultivation of rice has been reported to be less productive in high hills with the increase in altitude (Sthapit and Subedi 1990).

Nepal is rich in finger millet diversity at both the varietal and intrapopulation levels. As a result, finger millet was selected as one of the mandate crops across the three sites of the *in situ* conservation project (Baniya *et al.* 2003). Tiwari *et al.* (2003) reported 35 landraces under cultivation that were displayed during a diversity fair at Begnas. Diversity of finger millet is greater in the western and central regions. The mid-hill range crops finger millet in 75% of the area. Almost 33% of total finger millet areas and 44% of the area under mid-hills lies in the western region (Upreti 2003). This diversity could be further used for crop improvement. Thus, this study was carried out to study the intra- and interpopulation variation on finger millet landraces of Begnas, Kaski and Kachorwa, Nepal.

## Materials and methods

The experiment was conducted at the *in situ* site Begnas, Kaski (832 masl) in 2003. Landraces were selected on the basis of their popularity in the region and wide distribution in respective *in situ* sites of the study. Five landraces (*Arbali*, *Jhyape*, *Kukurkane*, *Shyangkhole* and *Seto dalle*) from Begnas, two (*Muna* and *Jhalari*) from Kachorwa and one improved (*Okhale-1*) as check were included in study.

Seeds were sown in a nursery bed and seedlings were transplanted in 1.0 m × 0.5 m plots with 10×10 cm spacing. All the cultural practices including land preparation, manuring and weeding were the same as farmers' practices. Thirty plants from each plot were studied for 12 qualitative and quantitative traits (Table 1) following the descriptors for finger millet (IBPGR 1985).

Rainfall, humidity and temperature for the period June to November were recorded in ARS (Fisheries) to represent the crop environment of Begnas, situated about 2.5 km from the experimental site.

Qualitative and quantitative observation data were processed in Excel for each landrace—mean value, range, coefficient of variation and standard error and percentage of different categories. Multivariate analysis (principal component and cluster analysis) was performed.

**Table 1. Qualitative and quantitative traits used in this study.**

Qualitative traits	Quantitative traits
Growth habit	Tiller/plant (n)
Ear shape	Leaf number (n)
Ear size	Leaf sheath length (cm)
Finger branching	Leaf blade length (cm)
Discontinuity of spikelets	Leaf blade width (cm)
Spikelet shattering	Blade length of flag leaf (cm)
Productive tiller	Blade width of flag leaf (cm)
Lodging susceptibility	Plant height (cm)
Synchrony	Ear exertion (cm)
Grain covering by glumes	Finger number
Grain colour	Finger length (cm)
Grain surface	Finger width (cm)
	1000-grain weight (g)
	Grain yield/plot (kg)
	Days to flowering
	Days to maturity

## Results and discussion

### Variation in quantitative traits

The value of mean, standard error (SE), range and coefficient of variation for most traits observed among eight landraces except 1000-grain weight, grain yield, days to flowering and days to maturity are presented in Table 2. Highest mean value for tiller number was found in *Kukurkane* and second largest in *Shyangkhole*. Maximum mean value for leaf number was observed in *Seto dalle* (18.5) followed by *Syangkhole* (17.1). Lowest mean value for this trait was observed in *Muna* (10.5) and *Jhalari* (10.3). Longest blade length of flag leaf was observed in *Kalo Jhyape* (27.1 cm) whereas *Muna* had the lowest (15.5 cm). *Jhalari* showed maximum variability (6.6%) whereas *Muna* showed less variability (3.4%) for blade length of flag leaf. *Muna* was the smallest of all (45.1 cm). *Seto dalle* had the highest finger number (6.4) but *Okhale-1* had long fingers (10.3 cm). *Jhalari* and *Muna* did not perform well for finger length and finger number.

Table 2a. Statistical parameters of finger millet landraces collected from Begnas, Kaski 2003.

Variable/Trait	Arbali kodo				Kalo Jhyape				Kukurkane				Seto Dalle				Syangkhole			
	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%
Tiller/plant	0.2±0.1	0-2	0.3	0.3	0.2±0.1	0.0-2.0	0.3	0.3	0.9±0.3	0.0-3.0	0.6	0.6	0.0	0.0	NA	NA	0.2	0.0-2.0	0.3	0.3
Leaf number	13.9±0.4	11.0-20.0	6.2	6.2	15.1±0.5	10.0-20.0	5.3	5.3	14.3±0.5	10.0-19.0	4.9	4.9	18.5±0.5	14.0-22.0	7.4	7.4	17.1±0.4	13.0-21.0	7.6	7.6
Leaf sheath length, cm	8.2±0.2	7.0-10.0	7.5	7.5	13.6±3.6	6.0-8.5	9.1	9.1	7.9±0.3	6.0-10.0	5.6	5.6	8.0±0.4	5.0-10.0	3.9	3.9	9.7±0.4	6.0-14.0	4.0	4.0
Leaf blade length, cm	34.7±0.8	27.0-42.0	8.4	8.4	34.6±0.8	29.0-42.0	7.6	7.6	29.6±0.5	25.0-35.0	11.9	11.9	38.0±1.0	27.0-48.0	6.7	6.7	33.9±0.7	29.0-42.0	9.5	9.5
Leaf blade width, cm	1.0±0.0	0.6-1.5	4.7	4.7	1.1±0.0	0.8-1.9	5.3	5.3	1.1±0.0	1.0-1.3	10.5	10.5	1.1±0.0	0.8-1.3	6.6	6.6	1.2±0.0	1.0-1.5	8.9	8.9
Blade length of flag leaf, cm	25.3±1.2	18.0-34.0	3.7	3.7	27.1±1.1	20.0-38.0	4.4	4.4	19.8±0.7	14.0-28.0	4.9	4.9	23.1±0.9	15.0-29.0	4.9	4.9	23.7±0.8	17.0-29.0	5.4	5.4
Blade width of flag leaf, cm	0.8±0.0	0.5-1.1	4.7	4.7	0.9±0.0	0.6-1.3	4.1	4.1	1.0±0.0	0.8-1.1	10.5	10.5	0.9±0.0	0.7-1.1	7.2	7.2	0.9±0.0	0.6-1.1	5.4	5.4
Plant height, cm	75.4±2.2	56.0-111.0	6.3	6.3	80.5±1.7	58.0-100.0	8.4	8.4	67.0±1.8	52.0-89.0	7.0	7.0	78.9±2.7	56.0-102.0	5.3	5.3	79.5±1.9	70.0-105.0	7.8	7.8
Ear extension, cm	14.0±0.5	10.0-19.0	5.2	5.2	14.7±0.5	10.0-19.0	5.3	5.3	11.4±0.3	9.0-14.0	6.8	6.8	9.0±0.9	3.0-17.0	1.9	1.9	8.3±0.3	6.0-12.0	4.4	4.4
Finger number	5.1±0.3	3.0-8.0	3.7	3.7	5.0±0.3	3.0-10.0	3.4	3.4	6.2±0.3	3.0-9.0	4.4	4.4	6.4±0.3	4.0-10.0	4.1	4.1	5.8±0.2	4.0-7.0	5.5	5.5
Finger length, cm	5.0±0.1	3.6-6.0	8.9	8.9	7.9±0.2	6.3-10.5	7.9	7.9	6.9±0.1	5.6-8.6	8.8	8.8	5.4±0.1	4.0-7.8	8.0	8.0	6.2±0.1	4.7-8.3	7.6	7.6
Finger width, cm	1.0±0.0	0.7-1.4	5.8	5.8	1.1±0.0	0.5-1.5	4.4	4.4	1.1±0.0	0.8-1.4	6.9	6.9	1.0±0.0	0.6-1.2	6.5	6.5	1.2±0.0	0.8-1.5	7.3	7.3
1000-grain weight	3.2	3.2	NA	NA	3.2	3.2	NA	NA	2.9	2.9	NA	NA	2.6	2.6	NA	NA	3.8	3.8	NA	NA
Grain yield/plot, kg	0.1	0.1	NA	NA	0.1	0.1	NA	NA	0.2	0.2	NA	NA	0.2	0.2	NA	NA	0.2	0.2	NA	NA
Days to flowering	115	NA	NA	NA	112	NA	NA	NA	112	NA	NA	NA	119	NA	NA	NA	109	NA	NA	NA
Days to maturity	155	NA	NA	NA	150	NA	NA	NA	155	NA	NA	NA	157	NA	NA	NA	149	NA	NA	NA

Table 2b. Statistical parameters of improved varieties and landraces collected from Kachorwa, Bara 2003.

Variables/Traits	Okhale-1				Muna				Jhalari			
	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%	Mean±SE	Range	CV%	CV%
Tiller/plant	0.0	0.0	NA	NA	0.0	0.0	NA	NA	0.0	0.0	NA	NA
Leaf number	14.4±0.4	11.0-18.0	7.4	7.4	10.5±0.5	9.0-14.0	7.3	7.3	10.3±0.4	9.0-13.0	8.2	8.2
Leaf sheath length (cm)	8.8±0.2	7.0-10.0	7.4	7.4	6.8±0.2	5.5-8.0	8.6	8.6	7.1±0.2	6.4-8.0	11.4	11.4
Leaf blade length, cm	33.9±1.0	25.0-43.0	6.0	6.0	22.8±1.1	18.0-30.0	6.3	6.3	25.5±0.8	21.5-29.5	10.4	10.4
Leaf blade width, cm	1.1±0.0	0.7-1.3	5.9	5.9	0.8±0.1	0.5-1.2	3.8	3.8	0.7±0.0	0.5-0.9	4.9	4.9
Blade length of flag leaf, cm	21.6±1.1	11.0-33.0	3.5	3.5	15.8±1.1	11.0-21.8	4.7	4.7	19.9±0.9	16.5-21.2	6.6	6.6
Blade width of flag leaf, cm	1.0±0.0	0.5-1.3	4.7	4.7	0.8±0.0	0.6-0.9	8.4	8.4	0.7±0.0	0.5-0.9	5.7	5.7
Plant height, cm	79.2±2.4	58.0-97.0	6.1	6.1	45.1±2.8	34.0-65.0	5.2	5.2	50.0±2.8	36.0-62.0	5.7	5.7
Ear extension, cm	9.9±0.7	3.0-15.0	2.6	2.6	6.7±0.4	5.0-8.0	5.5	5.5	9.8±0.7	6.2-12.3	4.3	4.3
Finger number	5.0±0.2	3.0-7.0	4.3	4.3	4.0±0.2	3.0-5.0	6.0	6.0	3.5±0.2	3.0-5.0	4.9	4.9
Finger length, cm	10.3±0.3	6.9-15.0	5.4	5.4	3.9±0.2	3.3-4.6	8.0	8.0	4.6±0.6	2.9-8.5	2.6	2.6
Finger width, cm	1.1±0.0	0.8-1.4	8.3	8.3	1.0±0.1	1.2-1.5	6.3	6.3	0.8±0.0	0.7-1.1	5.6	5.6
1000 grain weight	2.9	2.9	NA	NA	3.1	3.1	NA	NA	2.7	2.7	NA	NA
Grain yield/plot, kg	0.2	0.2	NA	NA	0.0	0.0	NA	NA	0.0	0.0	NA	NA
Days to flowering	107	NA	NA	NA	59	NA	NA	NA	59	NA	NA	NA
Days to maturity	144	NA	NA	NA	91	NA	NA	NA	91	NA	NA	NA

NA-Not available.

### Variation in qualitative traits

The summary of 12 qualitative traits observed among eight landraces is presented in Table 3. All landraces showed erectness in growth habit, continuation in spikelet on finger, low in lodging susceptibility, synchronous on ear maturity, enclosed and smooth grain surface. All landraces produced productive tillers. The largest ear size proportion was found in *Okhale-1* (36%) followed by *Seto dalle* and *Shyangkhole* (30%). Finger branching was observed more in *Kukurkane*, *Seto dalle*, *Shyangkhole* and *Okhale-1*. *Arbali kodo*, *Kalo jhyape*, *Muna* and *Jhalari* had no finger branching. Spikelet shattering was found to be 67% in *Okhale-1*, 12% in *Kalo jhyape* and 9% in *Kukurkane* and occurred in a lower proportion or was absent in other landraces. Spikelet shattering was not observed in *Arbali kodo*, *Syangkhole*, *Muna* and *Jhalari*. *Muna* was the dwarf among all landraces under study. Highest finger number (6.4) was found in *Seto dale* and *Okhale-1* had the longest fingers (10.3 cm). *Jhalari* and *Muna* were poor in finger number and shortest in length of finger.

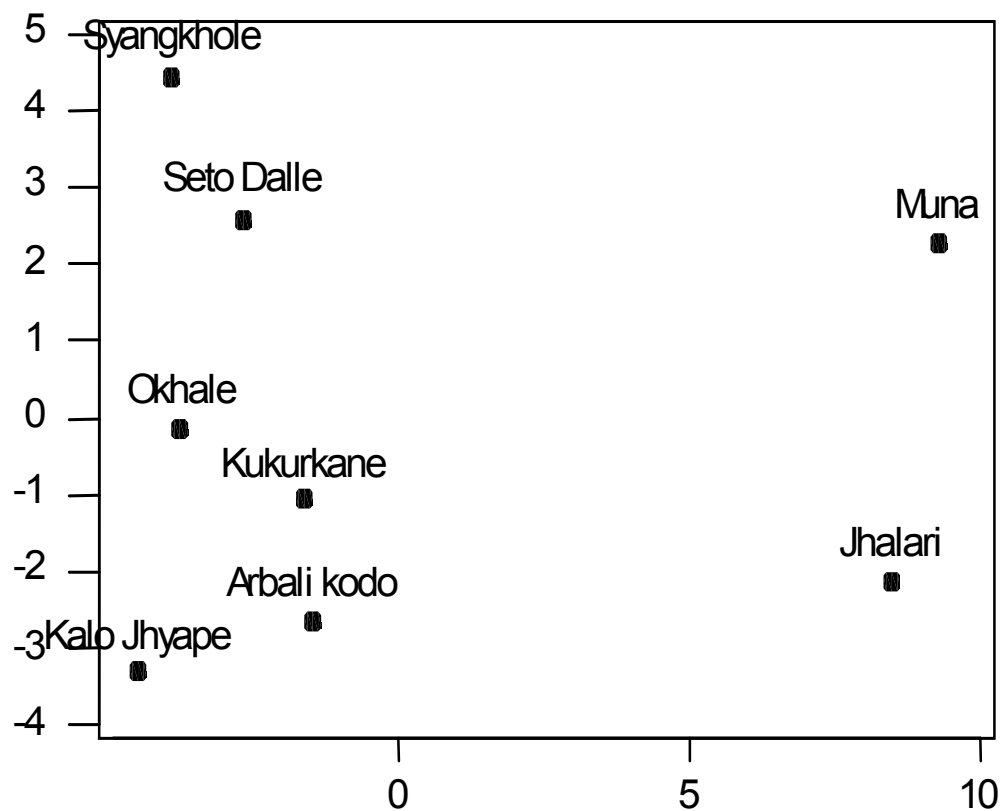
**Table 3. Frequency of variation in qualitative traits observed in finger millet landraces at Begnas, Kaski 2003.**

Trait		Landrace							
		Arbali kodo	Kalo Jhyape	Kukurkane	Seto Dalle	Syangkhole	Okhale-1	Muna	Jhalari
Growth habit	Erect	1	1	1	1	1	1	1	1
Ear shape	Fingers straight	0	1	1	0	0	0.09	0.1	0.4
	Tops of fingers curved	1	0	0	1	1	0.91	0.9	0.6
Ear size	Small	0.73	0.44	0.34	0.18	0.03	0.18	1	0.9
	Intermediate	0.27	0.4	0.46	0.52	0.67	0.46	0	0.1
	Large	0	0.16	0.2	0.3	0.3	0.36	0	0
Finger branching	Absent	1	1	0.67	0.67	0.6	0.97	0.8	0.9
	present	0	0	0.33	0.33	0.4	0.03	0.2	0.1
Discontinuity of spikelet on finger	Absent	1	1	1	1	1	1	1	1
Spikelet shattering	Absent	1	0.88	0.81	0.81	1	0.33	1	1
Productive tiller		1	1	1	1	1	1	1	1
Lodging susceptibility	Low	1	1	1	1	1	1	1	1
Synchrony of ear maturity	Synchronous	1	1	1	1	1	1	1	1
Grain covering by glumes	Enclosed	1	1	1	1	1	1	1	1
Grain colour	White	0	0	0	0	1	0	0	0
	Light brown	0.07	0.07	0	0.25	0	0	1	1
	Copper brown	0.93	0.93	1	0.75	0	1	0	0
Grain surface	Smooth	1	1	1	1	1	1	1	1

### Multivariate analysis of landraces

Principal component analysis was performed using all 15 quantitative characters and 77.8% of total variance is accounted for by the first two components. The first principal component accounted for 52.7% variance, and plant height and days to maturity were found most informative in describing the variability. Number of tillers, leaf number and blade length of flag leaf showed the maximum variability and accounted for 13.5% variability for the second component and discriminated the *terai* and hill landraces as distinct genotypes. Cluster

analysis grouped the improved check landrace along with the landraces from Begnas, Kaski. *Muna* and *Jhalari* were located separately (Figure 1). The depicted PC distribution plot shows the ecological grouping of the landraces.



**Figure 1. Scatter plot of landraces based on principal component analysis employing means of quantitative characters of finger millet.**

Landraces from Begnas have variability in five qualitative and seven quantitative traits. There was less variation in phenological traits such as days to flowering and maturity. The landraces included from Kachorwa were earlier than *Okhale-1* and landraces from Begnas, which may be due to environmental effect. The variability in finger branching, finger length and number of fingers could be useful for further research to improve yield potential. Maize-finger millet is the major cropping pattern in mid-hill agroecosystems of the country. Begnas, Kaski represents that region. The crop is a not given priority in terms of input and it is grown on the residual nutrient from the maize crop. As a result the performance of improved finger millet varieties was not desirable in low-altitude maize-millet system (Sthapit and Subedi 1990). However, studies show the local landraces to be more adaptive to the local environment and there could be effective results from improving these landrace genotypes. Within locally adopted germplasm, single plant progeny selection has been shown to be a highly effective method for rapidly improving self-pollinated crops (Baniya *et al.* 1993). These findings on inter- and intrapopulation variation could therefore be useful for further research in a varietal improvement programme.

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## Pigeon pea

## Population structure of pigeon pea (*Cajanus cajan* L. Millsp.) landraces

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### Abstract

Genetic diversity helps to mitigate the effects of environmental and socioeconomic challenges in production systems. Variation within landraces is necessary for adaptation to the given environment. The population structure of two landraces of pigeon pea was studied in Hardinath, Janakpur during 2001. Seeds of *Pajawa* and *Chanki* landraces were collected from 30 households of Kachorwa village development committee, Bara, 15 households for each landrace. A wide range of variation was exhibited among these populations of pigeon pea landraces for plant height, days to maturity, primary branching, seed number, 1000-grain weight and seed yield. Similarly, large variation with regards to stem colour, growth habit, plant habit, leaf pubescence and pod colour was observed within and between these landraces. Eye width and seed shape did not show variation in either landraces. *Chanki* showed variation in seed colour and seed colour pattern, whereas variation was noticed for base flower colour and pod shape in *Pajawa*. These variations are necessary to maintain better utilization of these landraces in diverse production environments and to meet the diverse needs of farmers and consumers.

**Key words:** Diversity, landraces, morphology, population structure, pigeon pea

### Introduction

Pigeon pea (*Cajanus cajan* L. Millsp.) is one of the most important legume crops in the Terai, Inner Terai and river basin of Nepal. The yield of pigeon pea remains static due to a lack of high-yielding varieties, which are basically cultivated on marginal land. It has many uses, for example seeds are used as *dhal*, the outer integument of seed together with part of the kernel are used as feed for cattle, the stalks of the plants are used for fuel, basket-making, roofing and fencing. Pigeon pea contains 20–21% protein and also provides all the essential amino acids required for human nutrition. It fixes nitrogen in soil to the extent of nearly 4070 kg/ha (Kumar Rao *et al.* 1981; Whiteman *et al.* 1985). The crop is grown in diverse ecological conditions with different cropping systems such as sole or intercropped with finger millet, maize or black gram in uplands. Cultivation in rice bunds is most common in Kachorwa, Bara, where many landraces are cultivated. Crop diversity and population structure are affected by farmers' selection of agromorphological traits and management (Jarvis and Hodgkin 1999).

Pigeon pea is one of the grain legumes growing in a larger area in Bara (Sherchand *et al.* 1998). The Nepal Agricultural Research Council has released two pigeon pea varieties suitable for low altitude (NARC 1998), although these improved varieties are not found in this site. Participatory rural appraisal and diversity fair indicated the existence of high landrace richness of pigeon pea in Kachorwa (Khatiwada *et al.* 2000). Farmers cultivate pigeon pea under low external input with no farmyard manure, chemicals or agrochemicals (Rana *et al.* 2000). Pigeon pea grains in the market fetch the highest price among the pulses (Rana *et al.* 2000). Pigeon pea, the only leguminous crop among the mandated crops of the *in situ* conservation programme, is often self-pollinated and a biennial crop. Because of its different breeding system we can relate population structure and breeding system with maintenance of diversity on farm. Farmers also use a small area to plant pigeon pea. In such a small population, if there is diversity then an understanding of population genetic



structure could help in policy formulation. Information on amount and distribution of diversity maintained by farmers on-farm is a prerequisite to effective implementation of in situ conservation activities. Therefore, this study was carried out to assess the population structure of pigeon pea landraces by growing them on-station.

### Materials and methods

The seeds of two pigeon pea landraces (*Pajawa* and *Chanki*) were collected randomly from 30 households of Kachorwa VDC, Bara in 2001. These two populations for which seeds were collected from 15 households for each were sown in the second week of May in rod row design without replication at National Rice Research Program, Hardinath. The research was conducted on-station about 170 km from the on-farm site. The cultural practices were done as per recommendation. Thiodan insecticide was sprayed at 2 ml/L of water twice at intervals of 15 days during the crop-growing season to protect against pod borer. Morphological (6 quantitative and 12 qualitative) traits were measured in 15 individual plants of each population following the methods described in IBPGR and ICRISAT (1993). Descriptive statistics were estimated and t-test was applied to separate the means. Frequency and Shannon-Weaver index (H') were calculated on qualitative traits as described by Tolbert *et al.* (1979). Data were processed and analyzed in Excel and SPSS software.

### Results and discussion

Population structure of two landraces (*Chanki* and *Pajawa*) with respect to six characters—namely plant height, days to maturity, primary branching, seed number, grain weight and seed yield—are given in Table 1. Plant heights had a mean of 238 cm in *Pajawa* and 219 cm in the *Chanki* populations. Coefficient of variation (CV) for plant height was higher in *Chanki*. *Pajawa* had higher CV for days to maturity with mean maturity days of 254. *Chanki* was earlier by 11 days on average. Average number for primary branching was 9.87 and 10.6 for *Pajawa* and *Chanki*, respectively. Variation was similar between these two populations for branching. The range for seed number per pod was the same in *Pajawa* and *Chanki* but CV was very high in *Pajawa*. CV of grain weight was very high in *Chanki*. yield per plant ranged from 18.6–31.5 g in *Pajawa* and from 14.3–22.9 g in *Chanki*. *Pajawa* yielded on average higher seeds than *Chanki*.

Statistically these two landraces are highly significantly different in plant height, days to maturity, 1000-grain weight and seed yield (Table 1). These landraces showed non-significant difference in primary branching and seed number per pod, which are yield components; however, yield was different between *Pajawa* and *Chanki*. So grain weight might have contributed relatively more to yield.

**Table 1. Variation in quantitative characters of pigeon pea landraces collected from Kachorwa, Bara, Nepal during 2001.**

Character	Pajawa				Chanki				P value
	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)	
Plant height (cm)	189-274	238	23.8	10.0	146-284	219	31.6	14.4	<0.001
Days to maturity	240-266	254	8.10	3.19	232-252	243	5.50	2.26	<0.001
Primary branching	9-12	9.87	1.13	11.4	9-13	10.6	1.35	12.7	0.094
1000-grain weight (g)	17.9-20.1	19.2	0.59	3.07	10.9-16.2	13.67	1.68	12.2	<0.001
Seeds per pod	2-4	3.6	0.63	17.5	2-4	3.5	0.74	5.4	0.745
Seed yield per plant (g)	18.6-31.5	26.29	4.09	15.5	14.3-22.9	17.98	2.66	14.7	<0.001

CV=Coefficient of variation; SD=Standard deviation.

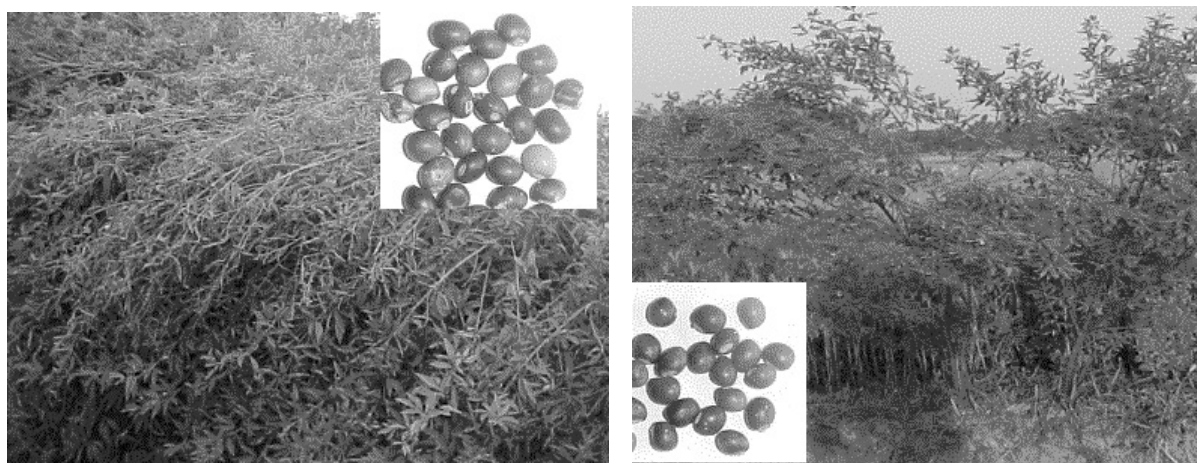
Plant vigour was predominantly very good followed by good in both these populations (Table 2). Plant growth habit was mostly erect and compact in *Pajawa* and spreading in *Chanki* (Figure 1). Intrapopulation variation in plant growth habits was greater in *Chanki*. Plant habit differed in both landraces from determinate to indeterminate. The variability was noticed within and between these populations for stem colour, leaf pubescence, pod colour and seed colour. There was no variation in base flower colour, pod shape, seed shape, eye width and seed colour pattern in *Chanki*. Likewise, seed colour pattern, seed colour, eye width and seed shape did not differ in *Pajawa*.

Shannon-Weaver index ( $H'$ ) of *Pajawa* was higher in plant vigour, plant habit, pod colour, base flower colour and pod shape than in *Chanki*. *Chanki* had higher  $H'$  in growth habit, seed colour and seed colour pattern than *Pajawa*. Both landraces showed similar index value in stem colour and leaf pubescence. Overall,  $H'$  was non significant between *Chanki* and *Pajawa*, which might be due to considering the homogenous traits.  $H'$  was also significantly different at household levels and between landraces in the study of Joshi *et al.* (2004).

**Table 2. Frequency of qualitative traits in two populations of pigeon pea landraces.**

Trait	Classes	Pajawa	Chanki	Shannon Weaver index ( $H'$ )	
				Pajawa	Chanki
Plant vigour	Very good	9	10	0.950	0.803
	Good	3	4		
	Poor	3	1		
Growth habit	Erect and compact	14	0	0.245	0.803
	Semi spreading	1	10		
	Spreading	0	4		
	Trailing	0	1		
Plant habit	Determinate	10	12	0.637	0.500
	Indeterminate	5	3		
Stem colour	Green	1	13	0.485	0.485
	Sunred	13	1		
	Purple	1	1		
Leaf pubescence	Glabrous	13	2	0.393	0.393
	Pubescent	2	13		
Pod colour	Purple	10	1	0.637	0.245
	Green and purple	5	14		
Base flower colour	Light yellow	14	15	0.245	0.000
	Yellow	1	0		
Seed colour	Purple	15	0	0.000	0.393
	Grey	0	13		
	White	0	2		
Pod shape	Flat	14	15	0.245	0.000
	Cylindrical	1	0		
Seed shape	Elongated	15	0	0.000	0.000
	Globular	0	15		
Seed eye width	Medium	15	0	0.000	0.000
	Wide	0	15		
Seed colour pattern	Plain	15	2	0.000	0.393
	Mottled and speckled	0	13		
Mean				0.320	0.335
SE				0.0895	0.0851

Seed samples of these landraces were collected from different households. It indicates that different households have grown the same landraces but with different variation in morphological traits. Joshi *et al.* (2004) also have reported diversity in pigeon pea landraces at household levels. Diversity also varied with respect to wealth category and production environments (Joshi *et al.* 2004). A farmer who has maximum diversity in pigeon pea is able to receive higher grain yield. Joshi *et al.* (unpublished) have reported that landrace populations of pigeon pea were genetically more diverse than improved varieties and, among these landraces, common landraces exhibited higher genetic diversity.



**Figure 1. *Pajawa* in uplands (left) and *Chanki* in rice-bunds (right).**

Relatively, *Chanki* expressed much more variation for both qualitative and quantitative traits. *Chanki* is a common variety grown by many households in larger areas (Rana *et al.* 2000). It is also grown in uplands and lowlands. Because of this variability in *Chanki*, it could be grown in diverse locations and could be suitable for various farming systems. The systems shape their present genetic structure and determine the changes within landrace populations (Brown 2000). Existence of variation within and between landraces probably was due to the often cross-pollinating nature, independent maintenance of seeds by each household, seed exchange, land fragmentation, etc. Variation in microclimate may be another important factor for creating variations.

Diversity is one of the basic criterion for breeders to use in improving crop yield and other traits. Knowledge of genetic diversity also helps to better manage genetic resources. The possibility exists to develop better varieties by utilizing variations found in these two populations. Owing to variation in lands, cultivation practices and farmers' and consumers' needs, the diversity within and between landraces should be maintained for long-term sustainable production. Farmers also consider quality in their continued cultivation of any landrace.

Numerous aspects of quality in pigeon peas are considered to be important nutritionally or economically. *Chanki* has high demand in the market because of its good taste of *dhal* soup, early maturity and other multiple use value, whereas the *Pajawa* landrace produced a high number branches and yield, including firewood (Rana *et al.* 2000). Farmers consider the market price in Nepal to be a function of seed colour (light red), taste, ease of cooking, swelling capacity and losses during milling. Genetics of the quality traits are, however, not measured systematically.

### **Conclusion**

The results indicate that these landraces are two distinct populations in Kachorwa. Within a small geographical area, farmers have maintained diversity. *Chanki*, a common landrace, showed more variability within-population in most of traits compared with *Pajawa*. Owing to this variability in *Chanki*, it could be grown in diverse locations and could be suitable for various farming systems. *Chanki* might have more cross-pollination and therefore it exhibited large variability in comparison with *Pajawa* and ultimately was adopted in different production systems. Considering a field as a population, the size of pigeon pea population is very small in some areas, which might lead to loss of genes. Therefore, a study to collect landraces from many fields and grow them in the same field, which allows reshuffling of genes and increasing population size from this experiment, is necessary for monitoring

population structure over time with respect to population size. Population study in terms of population size is also necessary for developing a strategy for on-farm management of agrobiodiversity. Because of often cross-pollinated crops, a gene flow study between and within landraces is important to further assess the factors that create variation. This could be useful to produce pure seeds and to develop a strategy for crop improvement.

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## Landrace-specific agronomic practices of pigeon pea (*Cajanus cajan* L. Millsp.) in Bara, Nepal

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### Abstract

Pigeon pea in Nepal covers approximately 25 015 ha and total production is 23 494 tonnes, with average productivity of 939 kg/ha. It is a rain-fed crop cultivated in marginal and submarginal lands and is considered the number one pulse crop in terms of its high quality preparation as *dhal* soup. A key informant survey was carried out during early July 2004 in Kachorwa, Bara in order to document different types of landraces of pigeon pea and their specific agronomic practices along with their postharvest care and storage methods and socioeconomic and cultural values. The various cultivation practices followed were sole cropping, mixed cropping with maize and black gram and bund cropping in rice fields that led to *in situ* conservation of landraces. *Chanki* and *Pajawa* are two landraces of pigeon pea with yield ranges of 0.5–1.0 and 0.6–1.0 t/ha, respectively. Likewise, dry stalk yields were in a range of 4.5–7.5 t/ha and 6.0–8.5 t/ha, respectively of *Chanki* and *Pajawa* landraces. We suggest further such study in other potential sites of Nepal. As well, yield improvement through agronomic management of the crop, plant protection measures and value-addition of the products through postharvest research and market promotion activities seem necessary in the near future.

**Key words:** Agronomic practices, landraces, pigeon pea, Nepal

### Introduction

Pigeon pea (*Cajanus cajan* L. Millspaugh) is an important pulse crop grown mainly in tropical areas, especially in South Asia, Eastern Africa, the Caribbean region, and South and Central America. It is known as red gram in South Asia, and about 90% of the world's pigeon pea is produced in India (Nene and Sheila 1990). In Nepal, it covers an area of 25 015 ha (8% of the total area under all grain legumes) with total production of 23 494 tonnes (9% of total production under all grain legumes) and average productivity of 939 kg/ha (MOAC 2002). It ranks third among grain legumes after lentil and black gram in Nepal. It is a rain-fed crop cultivated in marginal and submarginal lands of the *Terai* belts of Nepal (Joshi 1999). It has special significance among pulses in Nepal and is considered the number one pulse commodity in terms of its high quality preparation as *dhal* soup (the soup prepared either from the whole grains or from the split pigeon pea known as *dhal*). The split grain after removal of the husks during processing in the mill is called *dhal* in market. It is then a ready-made product for cooking *dhal* soup.

The importance of growing pigeon pea is highlighted in terms of household nutritional security and sustainability of agriculture (Joshi *et al.* 1998; Joshi 1999). Twelve landraces of pigeon pea were reported by early workers in Bara (Sherchand *et al.* 1998). Productivity of *Chanki* and *Pajawa* as reported earlier was 0.7 and 1.3 t/ha, respectively (Rana *et al.* 2000). Low stability of pigeon pea production leads to a high risk of genotype losses and substantial losses in storage happen quite often. Various ways of cultivating pigeon pea have been practised in Nepal since ancient times, such as sole upland crop, mixed crop with maize and bund crop in rice fields, which led to their *in situ* conservation. It is a rich source of protein, which is about 20–21.7%. Also, the chaff produced during threshing of the crop is a good source of dry fodder for livestock and the by-product from the *dhal* industry is an equally excellent source of protein for cattle. Farmers consider that sufficient foliage biomass left over the land where pigeon pea is grown improves the physical condition of the soil. Terminal pigeon pea branches with pods are carefully harvested and bottom stalks are then re-harvested and dried to use as fencing materials in the kitchen garden and/or as fuel.

Many of the local landraces cultivated throughout the *Tera*i region of the country are susceptible to wilt, sterility mosaic diseases and insects like *Helocoverpa* pod-borer, aphids and mites (Joshi *et al.* 1998; NGLRP 2002). Also, storage insects cause serious damage to the whole grains that are stored without being processed. However, previous studies have not taken into account of these facts. Therefore, a key informant survey was carried out in order to document pigeon pea based cropping patterns and crop husbandry practices along with existing farmer practices in its production, market values, marketing practices and storage systems.

### **Materials and methods**

A key informant survey of pigeon pea farmers composed of four groups at Ward No. 1, 5, 6 and seven of Kachorwa VDC in Bara was carried out in June 2004. A total of 40 farmers in four different groups, each having 8–10 farmers, was consulted. The sample included 50% representation of women farmers. Selection of the farmers was made on a random basis from within the command area of the *in situ* sites of Kachorwa, Bara. A Participatory Group Discussion approach was followed to get information on the predesigned questionnaires. Pertinent issues or concerns during group discussions were carefully noted during discussions with the key informant farmers. Information on quality aspects, marketing and storage methods and conditions were also rationally discussed with the farmers in order to find out their valuable knowledge and skills accumulated over generations. The information was supplemented with Baseline survey information.

### **Results and discussion**

#### **Cropping patterns**

There are three most common cropping patterns for upland pigeon pea cultivation, which are pigeon pea – fallow, pigeon pea + maize – fallow, and pigeon pea + black gram – fallow (Table 1). But there is only one pattern of pigeon pea – fallow followed in rice-bund pigeon pea. Both types—mixed cropping of maize with pigeon pea and black gram with pigeon pea—have gained popularity among farmers in recent years owing to a higher return than from the sole crop only.

#### **Yields**

*Chanki* and *Pajawa* are the two landraces being grown by the farmers in Kachorwa, Bara (Table 1) both for the sole cropping in rain-fed uplands and rice-bund cropping in rice fields. The contribution of local technology to enhancement of crop yields is an important factor that attracts the farmers to growing pigeon pea. Despite lower average yield of the crop, farmers have been growing the crop for a long time for many reasons. For instance, the crop is grown in marginal lands where other crops are not remunerative to grow. The average yields of *Chanki* under uplands and rice-bunds were slightly lower than those for *Pajawa*. Mean yields of *Chanki* were also slightly than those of *Pajawa*, as were the dry stalk yields (Table 1). Overall mean dry stalk yields in *Chanki* and *Pajawa* were 4.5–7.5 and 0.6–8.5 t/ha, respectively. There are additional benefits from stalk yields which are useful for many reasons such as green as well as dry fodder for cattle, fuel wood, dry stalks used for fencing, and dried stalks that are used for brooms. According to the farmers, slightly better results under rice-bund pigeon pea were obtained due to proper drainage conditions and the open environment of the rice fields that favoured the crop. *Chanki* is early maturing and has a better taste for *dhal* soup than *Pajawa*. *Pajawa* is a more branchy type that yields more grain and stalks yields than *Chanki*.

**Table 1. Pigeon pea types, cropping patterns, landraces and their agronomic practices at Bara.**

Item	Upland pigeon pea		Rice-bund pigeon pea	
Cropping pattern	1. Pigeon pea – Fallow 2. Pigeon pea + maize – Fallow 3. Pigeon pea + Black gram – Fallow		1. Pigeon pea – Fallow	
Landraces	Grain yield (kg/ha)	Stalk yield (kg/ha)	Grain yield (kg/ha)	Stalk yield (kg/ha)
• Chanki	0.4–1.0	4.0–6.0	0.6–1.0	5.0–9.0
• Pajawa	0.5–1.0	4.0–7.0	0.7–1.0	6.0–10.0
Land preparation, seed rate and sowing	Preparatory plowings done 2–3 times. Broadcasting of seeds done soon after the final plowing and then light planking is done to cover the seeds immediately after broadcasting to ensure better germination as well as protect the seeds from being eaten by the birds. The seed rate is 7–10 kg/ha. Spacing between plants were maintained approximately around 40–50 cm by thinning during weed control time. Sowing is done during first fortnight of June.		Direct dibbling of 2–3 seeds over the newly repaired rice bund with the help of a Khurpi (hand-tool) within the week of rice transplanting. Seed dibbling is done in two lines (one line each towards the edges of the bund, which is repaired every year with puddled soils. The seed rate is 3–5 kg/ha. Spacing between plants were maintained approximately around 30–40 cm between rows and around 20–25 cm between plants during weed control time. Sowing is done in July during rice planting but 2–3 days after transplanting of rice.	
Application of manures and fertilizers	In general, farmers are applying neither manure nor any fertilizers. However, about 400 kg of FYM is applied /ha by some farmers in recent years. Also, some are applying DAP at 30 kg/ha after final plowing of the field before sowing.		No fertilizers are applied to the bund culture of the crop.	
Weed control measures	In general, inter-row plowing is done once when the crop is at about one month stage. However, one weeding is mostly done once when the crop is at about one month stage. Thinning of excess plants is also done.		Usually, no weed control measure is practiced in the bund planted crop. But sometime one hand pulling of the luxuriantly growing weeds is done between 4–6 weeks stage of the crop age.	
Plant protection measures	Every year the problem of sterility mosaic is observed in few plants, which yields nothing. One of the widespread destructive diseases of pigeon pea is wilting caused by <i>Fusarium udum</i> Butler. No control measures followed by the farmers in mitigating diseases. For pod-borer problem some farmers are applying Endosol to protect the crop.		Every year the problem of sterility mosaic is observed in few plants, which yields nothing. One of the widespread destructive diseases of pigeon pea is wilting caused by <i>Fusarium udum</i> Butler. No control measures followed by the farmers in mitigating diseases. Even for the problem of pod-borer, no spraying is done to control the pest.	
Harvesting and threshing	Manual harvesting is done with the help of sickle when the crop fully matures. Depending upon the volume to handle, threshing is done manually by beating with stick or by bullocks or by tractors. Sunshine drying is done until the grains are safer to store.			
Postharvest care and storage	Storage methods are in earthen pots for grains and in jute sacks for dhal. Seed stored by treating with Malathion powder at 2 g/kg seed to protect from storage pests. Milling is done both by hand operated stone grinder (Janto) and by power operated mills to make Dhal which is less infested with storage insect-pests.			

### Agronomic practices

Modest agronomic practice is required for pigeon pea. Preparatory plowings are done 2–3 times for pigeon pea in upland culture. Broadcasting of seeds is done soon after the final ploughing, followed by a light planking to cover the seeds immediately after broadcasting to ensure better germination as well as to protect the seeds from being eaten-up by birds. The seed rate is 7–10 kg/ha. Spacing between plants was maintained at approximately 40–50 cm by thinning during weed-control time. Sowing is done during the first fortnight of June. Generally, farmers apply neither manure nor any fertilizers to pigeon pea. However, about 400 kg/ha of Farm Yard Manure (FYM) was applied by some farmers in recent years. Also, some of the farmers had applied di-ammonium Phosphate (DAP) at 30 kg/ha after final

ploughing of the field before sowing. In general, inter-row ploughing by bullocks to control weeds is done once when the crop is at about the 1-month stage.

Direct dibbling of 2–3 seeds is done over the newly repaired rice bund with the help of a *Khurpi* (hand tool) within a week of rice transplanting for rice-bund cropping of pigeon pea. Seed dibbling is done in two lines (one line each towards both the edges of the bunds that are repaired every year with puddled soils). The seed rate is 3–5 kg/ha. Spacing between plants was maintained at around 30–40 cm between rows and around 20–25 cm between plants during weed-control time. Sowing is done in July after 2–3 days of rice transplanting. No fertilizers are applied to the bund culture of the crop. However, one weeding is mostly done once when the crop is at about 1 month old for both plantings. Thinning of excess plants is also done during this stage. Every year the problem of sterility mosaic is observed in a few plants, which yield nothing. One of the widespread destructive diseases of pigeon pea is wilting caused by *Fusarium udum* Butler. No control measures are followed by the farmers in controlling diseases. However, for pod-borer problems some farmers applied Endosol to protect the crop.

Grains are used for preparing *dhal* (grains ground to make split *dhal*) in general and very often-green pods or grains are boiled and eaten. The price of the grain in current year ranged between Rs 30 and Rs 35/kg, whereas that of *dhal* was Rs 40–45/kg in the local market. Stalks were used as green and, to some extent, dry fodders for cattle, fuel wood, fencing and brooms.

Manual harvesting is done with the help of a sickle when the crop fully matures. Depending upon the volume to handle, threshing is done manually or by beating with a stick or by bullocks or tractors. Sun-drying of grains is done until the farmers are certain that the moisture percent of grains is reduced to a safe level for storage. Storage methods are earthen pots for grains and jute sacks for *dhal*. Seeds are stored after treating with Malathion powder at 2 g/kg of seed to protect them from storage pests. Milling is done both by a hand-operated stone grinder (*Janto*) and by power-operated mills to make *dhal*; the latter is less infested with storage insect-pests.

Seeds are made available through a barter system or are purchased from neighbours. One of the most perceived problems among pigeon pea growers was replacement of seed. Farmers were not much worried about seed replacement from outside districts. The farmers were little aware of the matters such as when to replace the seed, where to buy seed, where to get information about new seed, and when to replace adopted landraces, by exchanging or buying from neighbouring farmers. The most important system of seed distribution in existence is farmer-to-farmer, but the quantities distributed this way are not yet known.

Declining soil fertility as a consequence of intensive cultivation was well accepted by the farmers. However, the role/potential of legume crops to improve soil quality was less understood by the majority of farmers (>58%) at Kachorwa.

Farmers reported that they face more severe weed problems in upland conditions than in rice-bunds. Weeds and crops germinate together with the onset of the monsoon rains. Weeds grow much faster than the crop, and the cost of weed control is higher than the crop value in most cases. Therefore, farmers practise inter-row ploughing for weed control in pigeon pea as in the maize crop plots.

The bund crop method of pigeon pea improves surface drainage to avoid waterlogging during the rainy season. However, maintenance during transplanting time is important and expensive because rains invariably damage the bunds. Insect pests and diseases are important biotic constraints to crop production. However, farmers are not very attentive in their control measures in the present context, which might not permit them to save the crop in future.



## Conclusion

*Chanki* and *Pajawa* are two landraces that have been conserved for many years by the farmers in Kachorwa, Bara both as sole cropping in rain-fed uplands and as rice-bund cropping in rice fields. Although several cropping patterns are used in upland pigeon pea cultivation in Bara, only one pattern (Pigeon pea–Fallow) is followed in the rice-bund pigeon pea system. Mixed cropping with maize and black gram has been gaining popularity among farmers in recent years because of more return than from the sole crop. Despite lower average yield of the crop due to disease and insect infestation, farmers have been growing the crop for a long time for many reasons: it is easily grown, less labour intensive, low-input responsive, has high social and economic values. Therefore, value addition through public awareness and market promotion can promote cultivation by increasing demand in the community and outside. Farmers also face the problem of insect pests in storage. Barter system and purchase are the most common ways to secure seeds. Better agronomic management of pigeon pea could substantially improve the overall production and yields not only in Bara but also throughout Nepal.

Therefore, further studies on improving pigeon pea productivity and production through agronomic management of the crop, plant protection measures and value addition of the products through postharvest research and market promotional activities are suggested for the future.

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## Characterization of sponge gourd (*Luffa cylindrica* (L.) Roem) landraces of Nepal

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### Abstract

Diversity of sponge gourd as landraces under cultivation in different agroecosystems is a valuable resource for varietal improvement. Agromorphological characterization and their evaluation are the prime steps undertaken for conservation, utilization and improvement of these resources. Twenty-one sponge gourd landraces collected from Begnas (Kaski), Kachorwa (Bara) and landraces collected by Plant Genetic Resource Unit of Agriculture Botany Division, NARC were characterized on-station at the Agricultural Research Station, Malepatan, Pokhara. Landraces collected from each ecosite were based on their popularity and distribution within sites. Out of 18 qualitative traits, 7 were found invariable over landraces. Fruit shape and flesh flavour were observed as variable traits for all the genotypes. Three principal components accounted for 58% of total variance. The findings of this study would be useful for further research and varietal development programmes.

**Key words:** Characterization, diversity, genotype, invariant, landraces, sponge gourd

### Introduction

*Luffa* (*Luffa cylindrica* (L.) Roem), commonly known as sponge gourd, is a genus represented by 10 species; *L. cylindrica* (*L. aegyptica*), *L. actutangula* and *L. hermaphrodita* are three cultivated species, all with 26 chromosomes ( $2n=26$ ) (Dutt and Saran 1998). However, Joshi *et al.* (2004) reviewed the species, even those with varying 5 to 7 chromosomes. *Luffa cylindrica* and *L. acutangula* L. Roxb. are the domesticated species.

Among 50 domesticated vegetable crops in Nepal, sponge gourd is one of the important fruit vegetable crops, locally known as *Ghiraula*. It is a popular summer vegetable of Nepal and cultivated widely in-home gardens from *terai* to high hills during the rainy season. Mid-hill is the most diverse zone where farmers grow diverse vegetables including sponge gourd. Rana *et al.* (2000a) reported that among 206 sampled households, 188 (91.3%) grew sponge gourd in their farm and homestead in Kaski. The area under sponge gourd is rather small and only a few plants are grown in home garden for culinary purposes. However, production on a large scale is in practice in *terai*. Recently the crop has gained value with semi-commercial to commercial production in *terai* serving as an important agricultural item for income-generation for farmers at household level (Rana *et al.* 2000b). Unfortunately, no official statistics are available for area and production of sponge gourd and until recently no formal research has been undertaken with respect to conservation and utilization of sponge gourd in Nepal (Pandey *et al.* 2003).

The tender fruit is consumed as a fresh vegetable and the tender shoots are also consumed as green vegetables. The dried vascular system of the fruit is used as a bathroom sponge and is also a raw material for different industries.

Pandey *et al.* (2003) and Yadav *et al.* (2003) characterized sponge gourd landraces available in Begnas, Kaski and Kachorwa, Bara respectively. Local landraces are popular and widely grown in Nepal and they are important resources. Agromorphological characterization and their evaluation are the prerequisites for proper conservation and efficient utilization in improving the better varieties. In general, it is impracticable to characterize sponge gourd landraces on farmers' field owing to variable environmental conditions. Thus, this study was carried out to characterize the sponge gourd landraces of Nepal in a uniform environment.

### Materials and methods

Seeds of 22 landraces were collected for the study. Among them six were from the PGR Unit (ABD, NARC) collection and nine and seven were from *in situ* sites Begnas and Kachorwa respectively (Table 1). Diverse and popular landraces were prioritized during germplasm collection. Three farmer-named *Ujarka* landraces were included due to diversity in seed colour and were distinguished by adding the first letter of the farmer's name as a prefix (Table 2).

The experiment was conducted in ARS, Malepatan, Kaski (848 masl ) in a randomized block design with three replications in 2003. Seeds were directly sown at 50-cm depth in a pit filled with a mixture of organic manure and topsoil on 12 May 2003. Each plot consisted of three pits of each variety with 2.5 m row-to-row and 2.0 m plant-to-plant spacing. Each plant was topdressed to enhance vegetative growth with 10 g urea 45 days after sowing and was staked. All the cultural practices including land preparation, application of manure and weeding followed farmers' practices.

Twenty-six qualitative and five quantitative traits (Table 3) were studied for all plants following Joshi *et al.* (2004). Meteorological information of the site (temperature, rainfall and relative humidity) during the study period was recorded (Table 4). Data were analyzed using MS-Excel and MINITAB software.

**Table 1. Names of farmers, sources and location of sponge gourd collection.**

Landrace name	Farmer's name/Donor's name	Location
Hariyo Chhoto	Bhoj Raj Poudel	Begnas, Kaski
Hariyo Basaune	Debendra Adhikari	Begnas, Kaski
Hariyo Lamo	Krishna Maya Tiwari	Begnas, Kaski
Lamo Bose	Dev Raj Poudel	Begnas, Kaski
Hriyo Bose	Shiva Raj Subedi	Begnas, Kaski
Seto Bose	Bishnu Prasad Tiwari	Begnas, Kaski
Seto Lamo	Hari Maya Poudel	Begnas, Kaski
Seto Basaune	Dev Raj Poudel	Begnas, Kaski
Basaune	In situ Begnas	Begnas, Kaski
Jhingari	PGR, ABD (NARC)	Laxmipur, Udayapur
Toriya	PGR, ABD (NARC)	Garamani, Jhapa
Jangali Ghiraula	PGR, ABD (NARC)	Ratnapuri-1, Bara
Jhimni	PGR, ABD (NARC)	Ratnapuri-1, Bara
Sano Ghiraula	PGR, ABD (NARC)	Pipladi-6, Kanchanpur
Sagputti Ghiraunla	PGR, ABD (NARC)	Santapur-4, Rautahat
Harihar Tagwa Bhadaya	Sharada Devi Jaisawal	Kachorwa, Bara
Basmatiya	Rambha Devi	Kachorwa, Bara
Ujarka-B	Badri Prasad Kushwaha	Kachorwa, Bara
Lamka Ujarka	Pun Kumari Devi Gupta	Kachorwa, Bara
Hariharka	Maha Narayan Prasad yadav	Kachorwa, Bara
Ujarka-R	Raj Kali Devi Gupta	Kachorwa, Bara
Ujarka-N	Nisahara Khatoon	Kachorwa, Bara

**Table 2. Sponge gourd landraces used in the study.**

Landrace	Designated code	Landrace	Designated code	Landrace	Designated code
Basaune	Bs	Jhingani	Jhn	Hariharka	Hr
Hariharka Tagwa	HTB	Ujarka-B	U-B	Seto Bose	SBo
Bahdaiya					
Hariyo Lamo	HL	Lamka Ujarka	LU	Sano	SG
				Ghiraula	
Hariyo Chhoto	HC	Hariyo Bose	HBo	Ujarka-R	U-R
Seto Basaune	SB	Jangali	JG	Ujarka-N	U-N
		Ghiraula			
Sagputti Ghiraula	SaG	Lamo Bose	LBo	Setto Lamo	SL
Hariyo Basaune	HB	Toriya	To	Jhimni	Jh

**Table 3. List of traits used to characterize sponge gourd landraces.**

Qualitative traits			Quantitative traits
Cotyledon size	Shape of stem	Stem end fruit shape	Internode length
Cotyledon colour	Dorsal leaf pubescence	Fruit ribs	Stem thickness
Shape of stem	Ventral leaf pubescence	Fruit shape	Lateral shoots
Tendrils	Stem pubescence	Fruit colour	Petiole length
Leaf shape	Flower colour	Fruit skin texture	Peduncle length
Leaf size	Sex type	Flesh colour	
Leaf margin	Peduncle shape	Flesh flavour	
Leaf lobes	Peduncle separation from fruit	Seed colour	
Growth habit	Blossom end fruit shape	Sponge quality	

**Table 4. Weekly meteorological data of ARS (Hort) Pokhara, Kaski (during May–November 2003).**

	Max. temp (°C)	Min. temp (°C)	RH (%)	Precip. (mm)		Max. temp (°C)	Min. temp (°C)	RH (%)	Precip. (mm)
April 29– May 5	28.5	15.7	73.0	12.0	Aug 12– 18	32.4	22.9	97.0	19.3
May 6–12	29.3	13.8	63.0	47.9	Aug 19– 25	32.0	23.1	93.5	46.6
May 13– 19	29.4	15.9	77.0	17.1	Aug 26– Sept 1	31.4	22.7	91.0	41.0
May 20– 26	29.4	18.7	74.0	8.4	Sept 2–8	31.5	21.2	90.5	61.0
May 27– June 2	31.7	18.8	72.5	13.1	Sept 9–15	31.3	21.9	91.0	21.1
June 3–9	30.0	20.3	71.5	25.1	Sept 16– 22	30.3	21.9	88.5	39.6
June 10– 16	30.2	20.6	83.0	30.4	Sept 23– 29	28.5	19.4	87.5	13.9
June 17– 23	31.0	21.6	82.0	34.6	Sept 30– Oct 6	29.3	18.2	90.0	36.0
June 24– 30	29.7	21.8	91.5	46.8	Oct 7–13	29.1	18.4	85.5	0.0
July 1–7	31.2	22.0	93.0	60.1	Oct 14– 20	29.2	18.4	87.0	0.0
July 8–14	29.4	21.7	95.0	46.8	Oct 21– 27	27.9	14.6	92.5	0.0
July 15– 21	31.4	20.5	92.0	24.7	Oct 28– Nov 3	26.6	15.0	92.5	0.0
July 22– 28	33.1	22.4	95.5	38.0	Nov 4–10	25.1	14.7	91.5	27.4
July 29– Aug 4	30.9	22.3	85.5	24.0	Nov 11– 17	24.5	12.1	88.5	0.0
Aug 5–11	32.4	22.7	95.0	17.0					

## Results and discussion

### Characterization of qualitative traits

Out of 26 qualitative traits examined, 8 traits were invariant among the landraces under study. These traits were tendrils, growth habit, leaf margin, shape of stem, sex type, peduncle shape, flesh colour. A comparative performance of the landraces for qualitative traits is presented in Table 5. Out of 18 qualitative traits, *Sano ghiraula* found a single type for 12 traits and *Basaune*, *Sagupatti ghiraula* and *Ujarka-B* for 11 traits. About two-thirds of the genotypes were variable with one to many types for more than 50% of traits out of 18 variable traits. Most variability was observed in *Hariharka* (for 14 traits). *Ujarka-N*, *Seto boso*, *Seto lamo*, *Toriya* and *Hariharka tagwa bhadaiya* were variable with different types for 12 traits.

*Basaune*, *Hariyo boso*, *Hariyo chhoto* and *Hariyo lamo*, the landraces from Begnas, were more uniform for observed qualitative traits. In contrast, *Lamo boso* and *Seto lamo*, genotypes of Begnas, revealed maximum variability. Similarly, *Toriya*, *Jhingani* and *Jangali ghirula*—the landraces included from ABD, PGR unit—were found most variable for the traits examined. Among six landraces from Kachorwa, *Hariharka* was the most variable followed by *Ujarka-N* and *Hariharka tagwa bhadaiya*. On the otherhand, *Llamka Ujarka* and *Ujarka-B* were uniform for 12 and 11 traits respectively.

### Characterization of quantitative traits

The average value, standard error, range and coefficient of variation of quantitative traits: internodes length, stem thickness, lateral shoot number, petiole length and peduncle length are presented in Table 6. Maximum variability in internode length was found in *Hariyo chhoto* followed by *Ujarka-N*, *Hariharka* and *Seto basaune*. *Hariharka tagwa bhadaiya* was least variable in internode. Stem thickness was maximum in *Hariyo boso* and *Ujarka-B*, and least variable in *Jangali ghiraula*. Maximum number of lateral shoots was found in *Sano ghiraula* followed by *Jangali ghiraula* and *Jhimni*. *Lamo boso* and *Basaune* were less variable in number of lateral shoots. The most variability in peduncle length was observed in *Seto basaune* and *Sagupatti ghiraula*. *Basaune*, *Jhingni* and *Ujarka-B* were less variable.

Among landraces from Begnas, *Hariyo chhoto* and *Hariyo basaune* were the most and least variable in internode length, respectively. *Hariyo lamo* was more variable in lateral shoot number but least variable in petiole length. Greater variability in petiole length was observed in *Seto basaune*; *Basaune* was least variable.

*Jhimni* was most variable in internode length and *Sano ghiraula* had significantly more lateral shoots among all PGR collections. There was no significant difference in petiole length but more variability was observed in peduncle length in *Jangali ghiraula*.

Among six landraces included from Kachorwa, *Ujarka-N* and *Hariharka* were found variable in internode length. None of the landraces was significantly variable in lateral shoot number and petiole length. The greatest variability in peduncle length was observed in *Ujarka-R*.

### Multivariate analysis of landraces

The first three components showed 58% of total variance among landraces of sponge gourd for five quantitative traits (Table 7). It is obvious in the depicted ordination plot in Figure 1 that most landraces were clustered close to each other with some exceptional landraces (9, 10 and 14) and they were dispersed apart from each other. The principal component 2 found discriminating the group of landraces in cluster (Figure 2). Petiole length was observed an important trait in describing the variability among these landraces. Peduncle length has contributed negatively to all principal components. Likewise, lateral shoots positively contributed for the first and third components and petiole length contributed for the second principal component.

Table 5. Frequency data of qualitative traits of sponge gourd landraces characterized in Pokhara, Kaski 2003.

Characters	HC	HB	HL	LBo	HBo	Sbo	SL	SB	Bs	Jhn	To	JG	Jhm	SG	SgG	HTB	U-B	LU	Hr	U-R	U-N
Cotyledon size	0.6	1	-	1	0.3	0.7	0.6	0.7	1	1	1	1	0.7	1	1	0.6	0.7	1	1	1	0.8
Large	0.4	-	1	-	0.8	0.3	0.4	0.4	-	-	-	-	0.3	-	-	0.4	0.3	-	-	-	0.3
Cotyledon colour																					
Light green	-	-	0.38	0.5	-	-	-	-	0.6	0.8	-	-	0.7	1	1	1	-	-	-	0.1	-
Intermediate	1	1	-	0.3	-	0.1	0.7	1	0.4	0.3	0.9	0.2	0.3	-	-	-	1	1	1	0.9	1
Dark green	-	-	0.62	0.2	1	0.9	0.3	-	-	-	0.1	0.8	-	-	-	-	-	-	-	-	-
Leaf shape																					
Ovate	-	-	-	1	1	-	-	1	1	-	-	-	-	-	-	-	1	1	1	-	-
Orbicular	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	1	-
Reniform	1	1	1	-	-	1	1	-	-	1	-	1	-	-	-	1	-	-	-	-	1
Leaf size																					
Small	-	-	-	-	-	-	-	0.1	-	-	0.9	0.7	0.7	1	1	-	-	-	-	-	-
Intermediate	1	0.1	1	1	1	1	0.4	0.9	1	-	0.1	0.3	0.3	-	-	-	-	1	0.3	1	0.8
Large	-	0.9	-	-	-	-	0.6	-	-	-	-	-	-	-	-	1	1	-	0.8	-	0.3
Leaf lobes																					
Shallow	-	-	0.2	0.6	-	-	-	-	-	1	0.1	1	1	0.6	0.6	1	1	1	0.1	1	1
Intermediate	1	1	0.8	0.4	1	1	1	1	-	-	0.9	-	-	0.4	0.4	-	-	-	0.9	-	-
Deep	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Dorsal leaf pubescence																					
Absent	1	-	1	0.4	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	1	-
Low	-	-	-	0.6	-	1	-	-	1	0.7	-	-	-	1	1	0.5	1	1	0.8	-	1
Intermediate	-	1	-	-	1	-	-	1	-	0.3	1	-	1	-	-	0.5	-	-	0.3	-	-
Ventral leaf pubescence																					
Absent	1	-	-	-	-	0.7	1	-	0.2	0.7	0.1	-	0.7	-	-	-	1	-	-	1	0.1
Low	-	-	1	0.4	-	0.3	-	-	0.8	0.3	-	-	0.3	1	1	1	-	1	0.8	-	-
Intermediate	-	1	-	0.6	1	-	-	1	-	-	0.9	1	-	-	-	-	-	-	0.3	-	0.9
Stem pubescence																					
Absent	1	1	0.7	1	1	1	0.5	0.7	1	0.7	0.1	-	0.3	0.6	0.6	0.8	1	1	1	1	1
Thin	-	-	0.3	-	-	-	0.5	-	-	-	0.9	1	0.7	0.4	0.4	0.3	-	-	-	-	-
Dense	-	-	-	-	-	-	-	0.3	-	0.3	-	-	-	-	-	-	-	-	-	-	-
Peduncle separation from fruit																					
Easy	0.9	0.9	1	1	1	0.8	0.7	1	1	0.3	1	0.8	-	0.3	0.3	0.8	0.8	1	0.8	0.3	0.8
Intermediate	-	0.1	-	-	-	0.2	-	-	-	-	-	0.2	-	-	-	-	0.1	-	-	0.1	-
Difficult	0.1	-	-	-	-	-	0.3	-	-	0.8	-	-	1	0.8	0.8	0.2	0.1	-	0.3	0.6	0.3
Blossom end fruit shape																					
Depressed	-	-	0.1	-	-	-	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-
Flattened	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	-	-	-	-	-	-	0.1	-
Rounded	1	0.2	0.3	0.6	0.1	0.4	0.1	0.4	-	-	0.9	0.3	0.7	0.7	0.7	0.6	-	0.3	0.5	-	0.3
Pointed	-	0.8	0.6	0.4	0.9	0.6	0.9	0.6	1	0.3	0.1	0.6	0.2	0.3	0.3	0.4	1	0.7	0.5	0.9	0.8
Stem end fruit shape																					
Rounded	0.4	0.1	0.4	0.6	-	0.3	0.1	-	-	-	0.7	0.5	-	-	-	0.2	-	0.4	0.5	0.1	0.4
Pointed	0.6	0.9	0.6	0.4	1	0.8	0.9	1	1	1	0.3	0.5	1	1	1	0.8	1	0.6	0.5	0.9	0.6

Table 5. (cont'd.)

	Characters	HC	HB	HL	LBo	HBo	Sbo	SL	SB	Bs	Jhn	To	JG	Jhm	SG	SgG	HTB	U-B	LU	Hr	U-R	U-N
Fruit shape	Oblong blocky	0.1	-	-	-	-	-	-	-	-	0.7	0.1	0.2	0.3	0.7	0.7	-	-	-	-	0.1	-
	Elongate slim	-	0.1	0.4	0.8	-	0.2	0.7	-	0.6	-	-	-	0.3	-	-	-	-	0.3	-	-	-
	Elongate blocky	0.9	0.4	0.4	0.2	0.6	0.3	0.1	0.5	0.2	-	0.9	0.8	-	-	-	0.6	0.6	0.1	0.8	0.3	0.9
	Elliptical	-	-	-	-	0.4	0.2	-	-	-	-	-	-	-	-	-	-	-	0.6	0.1	0.4	-
Fruit ribs	Elongate tapered	-	-	-	-	-	-	-	-	-	0.3	-	-	0.3	0.3	0.3	-	-	-	-	-	-
	Pyramiform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
	Elongate elliptical	-	0.4	0.3	-	-	0.3	0.1	0.5	0.2	-	-	-	-	-	-	0.4	0.4	-	0.1	-	0.1
	Superficial	-	0.1	-	-	-	-	-	-	-	1	-	-	1	1	1	-	-	-	0.1	0.1	-
Fruit colour	Intermediate	0.6	0.7	0.4	0.4	0.6	0.4	0.7	0.2	1	-	-	0.7	-	-	-	0.6	0.4	-	0.4	0.8	0.9
	Deep	0.4	0.2	0.6	0.6	0.4	0.6	0.3	0.8	-	-	1	0.3	-	-	-	0.4	0.6	1	0.5	0.1	0.1
	Light green	0.1	0.4	0.1	-	0.6	0.2	0.1	0.2	0.2	1	0.1	-	1	1	1	-	-	-	0.3	0.3	0.4
	Dark green	0.9	0.6	0.9	0.8	0.1	0.4	0.9	0.3	0.8	-	-	1	-	-	-	0.3	-	-	0.5	-	0.7
Fruit skin texture	White mottled	-	-	-	0.2	0.3	0.3	-	0.4	-	-	0.9	-	-	-	-	0.8	1	1	0.3	0.8	-
	Smooth	0.1	0.2	-	0.6	1	-	-	0.2	-	0.7	0.1	0.5	1	1	1	0.2	-	-	0.1	0.3	0.4
	Grainy	0.9	0.8	1.0	0.4	-	1	1	0.8	1	0.3	0.9	0.5	-	-	-	0.8	1	1	0.9	0.8	0.6
	Insipid	0.1	0.4	0.3	0.2	0.3	-	0.3	0.3	0.4	-	-	-	-	-	-	0.4	0.4	0.4	0.4	0.3	0.4
Flesh flavour	Intermediate	-	-	0.3	0.2	0.1	-	-	0.2	-	0.3	0.3	0.3	0.7	0.3	0.3	0.2	0.3	0.1	-	0.4	-
	Bitter	-	-	-	-	-	-	-	-	-	0.7	0.7	0.7	0.3	0.3	0.3	-	-	-	-	-	-
	Favourable	0.9	0.6	0.5	0.6	0.6	1	0.7	0.4	0.6	-	-	-	-	0.3	0.3	0.4	0.4	0.4	0.6	0.4	0.6
	Black	1	1	1	1	0.5	0.3	0.3	0.2	-	-	0.4	1	-	-	-	-	0.2	0.3	0.8	1	0.3
Seed colour	Gray	-	-	-	-	0.5	0.2	0.2	0.2	0.3	-	0.3	-	-	-	-	-	0.8	0.1	0.1	-	0.3
	Brown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	0.1	-	0.7
	Dark black	-	-	-	-	-	0.5	0.5	0.6	0.8	1	0.3	-	1	1	1	1	-	0.3	-	-	-
	Soft	0.1	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.8	1	0.6	0.8	1	1	1	0.4	0.3	0.4	0.4	0.3	0.4
Sponge quality	Intermediate	0.9	0.7	0.8	0.6	0.8	0.7	0.7	0.7	0.2	-	0.4	0.3	-	-	-	0.6	0.7	0.6	0.6	0.8	0.6

Table 6. Statistical parameters of sponge gourd landraces, Pokhara, Kaski 2003.

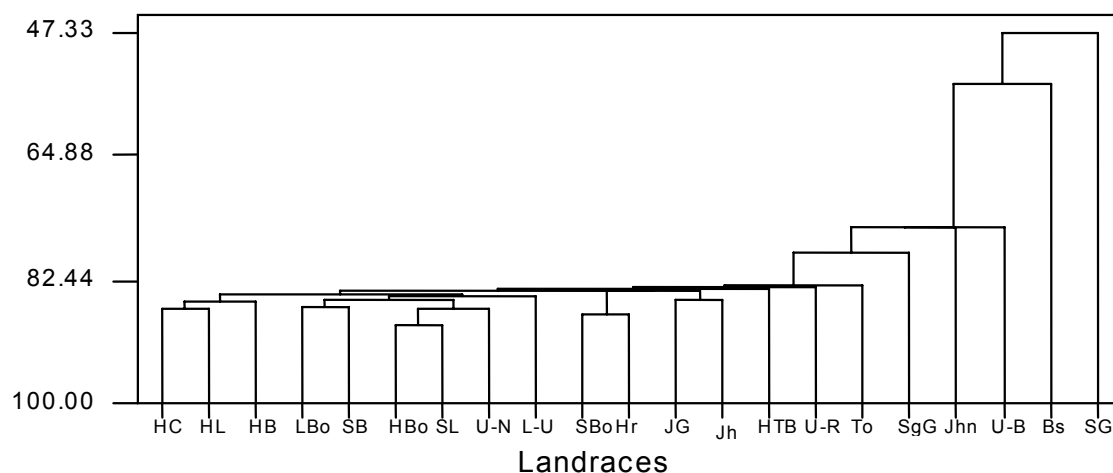
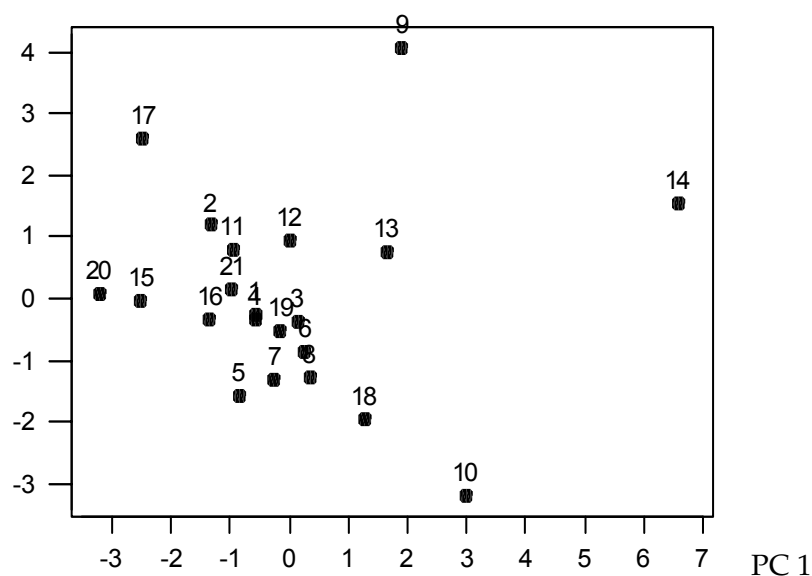
Landrace	Internode length, cm			Stem thickness, cm			Lateral shoots, no.			Petiole length, cm			Peduncle length, cm		
	Mean±SE	Range	CV%	Mean±SE	Range	CV%	Mean±SE	Range	CV%	Mean±SE	Range	CV%	Mean±SE	Range	CV%
Hariyo Chhoto	13.4±1.4	8.5–19.3	3.5	4.8±0.25	4.0–6.0	6.8	8.5±1.8	3.0–16.0	1.7	10.2±0.86	7.5–13.3	4.2	9.5±1.11	4.0–13.7	3.0
Hriyo Basaune	14.0±0.5	11.5–15.5	10.8	4.7±0.33	3.0–6.0	4.7	10.2±1.51	3.0–16.0	2.3	11.1±0.77	5.9–13.3	4.8	12.0±1.08	6.0–17.0	3.7
Hariyo Lamo	13.7±0.7	12.6–18.0	7.0	4.6±0.26	3.0–5.0	6.2	10.0±2.22	2.0–20.0	1.6	8.2±0.67	5.6–10.5	4.3	12.0±1.0	7.0–16.0	4.3
Lamo Bose	14.9±1.6	10.8–18.2	4.6	5.0±1.0	4.0–6.0	3.5	8.4±1.03	5.0–11.0	3.7	10.1±1.36	6.8–14.0	3.3	7.3±1.78	4.0–11.5	2.0
Hariyo Bose	14.8±1.1	11.2–19.0	5.0	5.0±0.42	3.0–6.0	4.2	10.3±1.7	5.0–20.0	2.1	8.5±0.64	5.6–11.1	4.7	12.1±1.0	8.0–17.5	4.3
Seto Bose	12.7±0.8	9.2–15.5	5.6	5.2±0.28	4.0–6.0	6.3	8.0±1.67	2.0–19.0	1.6	9.7±0.95	6.2–15.0	3.6	12.9±1.43	7.5–19.0	3.2
Seto Lamo	16.3±2.8	12.8–20.0	5.8	4.1±0.13	4.0–5.0	11.7	7.3±2.35	2.0–19.0	1.2	12.9±1.34	10.0–18.8	3.9	15.8±2.43	9.5–27.0	2.5
Seto Basaune	16.3±1.3	11.5–21.7	4.2	4.7±0.29	4.0–6.0	5.4	11.2±1.43	4.0–19.0	2.6	10.7±0.96	5.8–13.7	3.9	16.0±2.24	6.0–28.0	2.4
Basaune	15.6±1.0	13.8–18.5	7.8	4.8±0.25	4.0–5.0	9.6	6.8±0.97	4.0–10.0	3.1	8.4±0.89	6.2–12.1	3.9	11.2±0.9	8.0–13.0	5.5
Jhinagni	15.6±2.2	13.3–17.8	4.1	3.7±0.33	3.0–4.0	6.4	4.0±7.02	6.0–28.0	0.3	8.0±1.68	5.0–10.8	2.7	7.7±1.65	6.0–11.0	2.7
Toriya	15.7±0.7	13.2–18.9	8.3	4.2±0.37	3.0–5.0	5.0	10.0±1.69	3.0–17.0	2.2	7.3±0.53	5.9–10.0	5.2	9.3±0.75	6.0–12.0	4.7
Jangali Ghiraula	13.6±0.86	11.7–17.5	6.5	4.1±0.16	4.5–4.0	20.5	9.0±3.96	1.0–28.0	0.9	9.9±1.17	5.8–15.7	3.2	12.9±2.56	5.5–22.5	2.1
Jhimni	13.0±3.24	7.2–18.4	2.3	4.5±0.5	5.0–4.0	6.4	14.0±7.51	6.0–29.0	1.1	12.4±0.92	9.9–14.3	6.8	7.2±1.36	4.5–9.0	3.1
Sano Ghiraula	12.7±2.27	8.5–16.3	3.2	3.5±0.5	4.0–3.0	5.0	26.3±10.93	13.0–48.0	1.4	6.8±0.8	5.4–8.2	4.9	6.5±0.74	5.0–7.4	5.0
Sagputti Ghiraula	15.6±0.83	12.2–18.7	6.6	4.3±0.16	4.0–5.0	9.3	10.4±1.38	3.0–16.0	2.6	8.0±0.67	4.8–10.3	4.2	6.8±0.57	4.0–8.5	4.2
Harihar Tagwa Bhadaiya	13.91±0.67	13.3–14.5	15.6	5.0±0.27	4.0–6.0	6.6	5.2±1.77	2.0–10.0	1.3	10.6±0.98	8.0–13.1	4.8	9.3±1.78	3.0–14.0	2.3
Ujarka-B	14.0±0.89	10.4–17.8	5.2	4.6±0.38	3.0–6.0	4.1	5.9±1.39	1.0–15.0	1.4	10.7±0.87	7.3–15.5	4.1	9.5±0.59	7.0–12.0	5.4
Lamka Ujarka	15.5±0.67	13.5–18.3	9.5	4.1±0.26	3.0–5.0	5.3	6.8±2.4	2.0–14.0	1.3	9.8±0.55	8.8–11.1	9.0	13.4±2.26	6.5–19.5	2.2
Hariharka	13.8±1.05	8.8–19.0	4.6	4.4±0.37	3.7–5.0	6.7	5.9±1.18	1.0–10.0	2.3	9.2±0.98	5.8–12.0	3.3	10.0±0.97	5.7–15.0	3.6
Ujarka-R	14.4±0.66	11.2–16.3	8.4	4.3±0.2	4.0–4.7	12.3	7.8±1.45	2.0–16.0	3.5	10.5±0.79	7.3–13.8	2.3	12.0±1.62	3.8–17.3	2.6
Ujarka-N	18.1±1.25	13.2–23.8	5.1	5.6±0.37	5.0–6.3	8.6	8.7±1.29	2.0–12.0	8.7	9.4±0.53	7.2–12.0	3.7	13.1±0.91	10.5–18.0	5.1



**Table 7. Variance and values for five quantitative characters of sponge gourd.**

Variables	PC 1	PC 2	PC 3
Internode length	-0.40	0.00	-0.17
Stem thickness	-0.80	0.03	0.21
Lateral shoots	0.87	0.22	-0.69
Petiole length	-0.64	0.69	0.21
Peduncle length	-0.90	-0.14	-0.69
Eigen value	3.01	1.19	0.53
Variance %	30	16.8	13

Similarity

**Figure 1. Dendrogram showing the clustering patterns of 21 landraces of sponge gourd.**

**Figure 2. Plots of principal component based on 5 quantitative characters for 21 sponge gourd landraces, where: 1=Hariyo Chhoto; 2=Hariyo Basaune; 3=Hariyo Lamo; 4=Lamo Bose; 5=Hariyo Bose; 6=Seto Bose; 7=Setto Lamo; 8=Seto Basaune; 9=Basaune; 10=Jhingani; 11=Toriya; 12=Jangali Ghiraula; 13=Jhimni; 14=Sano Ghiraula; 15=Sagputti Ghiraula; 16=Harharka Tagwa Bhadaiya; 17=Ujarka-B; 18=Lamka Ujarka; 19=Harharka; 20=Ujarka-R; 21=Ujarka-N.**

Farmers use morphological traits to distinguish landraces (Table 8). This is also the common trend of farmers in Begnas and Kachorwa. Phenotypic traits like colour of fruit and size of fruit are commonly used in identification and characterization of the landraces of sponge gourd. For example, *Seto* (light green), *Black* (dark green), *Lamo* (long fruit), *Chhoto* (short fruit) are some landraces distinguished by farmers as distinct land types. Farmers also use most often the preferred and non-preferred traits as descriptors for sponge gourd diversity on-farm (Rana *et al.* 2000b).

**Table 8. Preferred and non-preferred traits of sponge gourd in Kaski and Bara.**

Site	Preferred traits	Non-preferred traits
Kaski	High yield	Less fruit setting
	Good taste	Poor taste
	Less spongy for longer time	Early development of sponginess
	Long harvest period	Less amount of seed
	Early fruiting	Watery when cooked
Bara		Late fruiting
	High yield	Insect disease incidence
	Good taste	Early development of sponginess
	Soft when cooked	Undesired colour after cooking
	Early fruiting	Late fruiting
	Long harvest period	
	Tasty curry	

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## Evaluation of sponge gourd (*Luffa cylindrica* L.) diversity for vegetable production

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### Abstract

Evaluation of landraces is necessary for better management of agricultural biodiversity on-farm. Their performances over the seasons and locations determine whether they will be cultivated or not. The objective was to evaluate the farmer-named landraces of sponge gourd that are being maintained on-farm for tender fruit production. A total of 21 sponge gourd landraces collected from Begnas Kaski, Kachorwa Bara and Agriculture Botany Division, Khumaltar were evaluated in a randomized complete block design with three replications at the Agriculture Research Station, Malepatan, Pokhara (900 masl) in 2003. Each plant was supported by dead branches of guava trees, the traditional practice for supporting vines. The landraces/accessions were evaluated for 11 traits related to fruit vegetable production. Minimum temperature and relative humidity were higher during the harvesting period of fruits for vegetable purpose. Large variation was observed in phenological and fruit traits. Fruit was harvested earliest (80 days) from *Jhingani* whereas *Toriya* produced fruits up to 169 days after seeding. *Toriya* had the longest fruit-harvesting period (67 days), which seems to be a good option for a home garden. *Hariyo Lamo* had the longest fruit (29.45 cm) and *Seto Basaune* produced the heaviest fruit (269 g). Highest number of fruits was harvested from *Sano Ghiraula* (74.6), but yield was highest from *Seto Basaune* (4606 g). Last harvest days, harvesting period and fruit number/plant have shown a strong relationship with fruit yield. Variation for tender fruits among these landraces can be utilized for improving the genotypes of sponge gourd as well as for long-term production in diverse environments.

**Key words:** Evaluation, fruit vegetable, *Luffa cylindrica*, sponge gourd landraces

### Introduction

*Luffa* (*Luffa cylindrica* (L.) Roem Syn. *L. aegyptiaca* Mill.), commonly called sponge gourd, loofah or dishcloth gourd, is a member of the Cucurbitaceae family, of which two cultivated species (*L. cylindrica* and *L. acutangula*) and one wild species (*L. echinata*) occur in Nepal. The Agriculture Botany Division (ABD) Khumaltar has conserved 60 accessions of sponge gourd (Gupta *et al.* 2000). Nepalese farmers call *L. cylindrica* *Ghiraula*. It is a summer-season vegetable grown from Terai to high hills in Nepal. Mid-hill is the most important zone where most of the farmers grow sponge gourd.

Farmers use living tree, dead branches, a wall or roof for supporting the climbing vines. The crop is cross-pollinated and therefore insect pollinator is necessary for better fruit production. The flowers are produced in the leaf axil with 4 to 20 staminate flowers and one pistillate flower in the same axil. The flower opens in the early morning and remains open only for one day (Porterfield 1955). *Luffa cylindrica* is only monoecious (Singh 1958) but other species of *Luffa* have four types of inflorescences: monoecious, andromonoecious, gynoeceous and hermaphroditic (Davis 1996).

It has multiple use values. Immature fruit is used as a vegetable whereas mature sponges are used for cleaning utensils and in the bathroom. Fresh juice of the leaf is used for healing wounds and also used as primer in door and windows by Nepalese farmers. Dried sponge, which is fibrous, is used in commercial filters and for insulation (Porterfield 1955). The seeds yield a colourless, odourless, tasteless oil that can be used in cooking (Porterfield 1955).

Despite its importance as a vegetable by the Nepalese people, limited efforts have been made to improve sponge gourd. Only one variety—*Kantipure*—has been released in Nepal (NARC 2000). Diversity of landraces and farmers' preferred traits for sponge gourd were studied by Rana *et al.* (2000a, 2000b). Based on diversity of Nepalese landraces, descriptors

for sponge gourd were developed by Joshi *et al.* (2004). On-farm agromorphological characterization was done by Bajracharya *et al.* (1999), Pandey *et al.* (2003) and Yadav *et al.* (2003). Cruz *et al.* (1997) studied correlation and variability using morphological and biochemical characters.

The existence of many landraces indicates that there is great possibility to increase yield through improvement in genetic and agronomic practices. Some landraces are better for sponge and others are good for fruit vegetables. Landraces are adapted to either tropical or subtropical to temperate climates. After on-farm characterization of these landraces, we should evaluate them for better utilization. As genetic erosion of landrace diversity is increasing, evaluation has become a necessity for on-farm and *ex situ* conservation, at least for most the important types. The primary objective of this study was to evaluate landraces collected from different areas of Nepal for tender fruits that can be used as vegetable.

### **Material and methods**

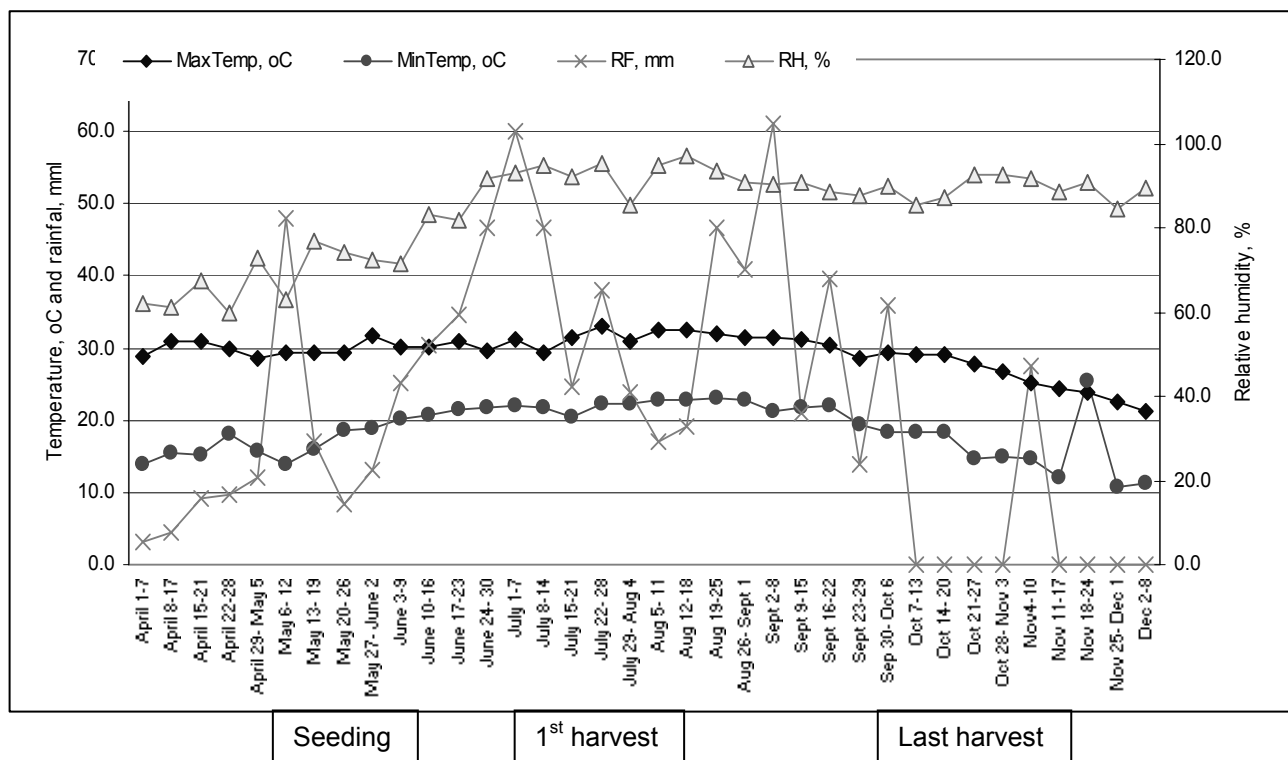
A total of 21 landraces, collected from Begnas Kaski, Kachorwa Bara and ABD Khumaltar were evaluated (Table 1). All landraces available in Begnas and Kachorwa were collected from farm stores. Effort was made to select diverse landraces from the Plant Genetic Resources Unit, ABD collections. In Bara, seeds of many accessions of *Ujarka* landrace were collected. Owing to diversity in seed colour, three types in *Ujarka* were used and their names were prefixed with the initial of the name of the farmer who provided the material. Some of them have been characterized on-farm by Pandey *et al.* (2003) and Yadav *et al.* (2003).

The experiment was conducted in a randomized complete block design with three replications at the Agriculture Research Station, Malepatan, Pokhara. The experimental site (Malepatan) lies between latitude 28°15'N and longitude 84°00'E at an altitude of 848 m in a subtropical area having loam soil. Agroecologically, Malepatan and Begnas are similar. Three to five plants of each landrace were maintained in each plot. Seeds were sown directly in pits containing 3 kg compost on 12 May 2003. Plants were spaced at 2.5 × 2.0-m. For the expected growth, 10 g urea/plant was topdressed at 1.5 months after seeding. All other agronomical practices were as used in the farmers' systems. *Basmatiya* did not germinate and it was excluded from analysis and reporting. Each plant was supported by a dead tree branch. Manual weeding and guarding were done to protect the fruits from weeds and birds.

Climatic parameters (temperature, rainfall and relative humidity) during the growing period were recorded and analyzed (Figure 1). Eleven traits related to tender fruits production were recorded as described by Joshi *et al.* (2004). Details of traits studied are summarized in Table 2. Sample size ranged from 3–5 fruits and/or 3–5 plants. Three fruits from each plant were left for seed production; therefore actual tender fruit yield was less by 3 fruits. Self-fed seeds of each landrace were given to the Plant Genetic Resources Unit, ABD, NARC, Khumaltar for *ex situ* conservation. Data were subjected to multivariate analysis of variance (MANOVA), analysis of variance (ANOVA) and mean separation (DMRT). Mean, standard deviation (SD) and probability (*P*) values were presented. Correlation coefficients were estimated among these traits. Data were processed in MS Excel and analyzed in MINITAB and MSTATC.

**Table 1. Landraces of sponge gourd evaluated in Malepatan, Pokhara.**

Landrace	Farmer's /donor's name	Collection site	Altitude (m)
Basaune	Diversity fair	Begnas, Kaski	848
Basmatiya <sup>†</sup>	Rambha Devi	Kachorwa, Bara	85
Harihar Tagwa Bhadaiya	Sharada Devi Jaisawal	Kachorwa, Bara	85
Hariharka	Maha Narayan Prasad Yadav	Kachorwa, Bara	85
Hariyo Basaune	Debendra Adhikari	Begnas, Kaski	848
Hariyo Chhoto	Bhoj Raj Poudel	Begnas, Kaski	848
Hariyo Lamo	Krishna Maya Tiwari	Begnas, Kaski	848
Hariyo Bose	Shiva Raj Subedi	Begnas, Kaski	848
Jangali Ghiraula	PGRU, ABD NARC <sup>‡</sup>	Ratnapuri-1, Bara	250
Jhimni	PGRU, ABD NARC	Ratnapuri-1, Bara	250
Jhingani	PGRU, ABD NARC	Laxmipur, Udaypur	–
Lamka Ujarka	Pun Kumari Devi Gupta	Kachorwa, Bara	85
Lamo Bose	Dev Raj Poudel	Begnas, Kaski	818
Sagputti Ghiraula	PGRU, ABD NARC	Santapur-4, Rautahat	300
Sano Ghiraula	PGRU, ABD NARC	Pipladi-6, Kanchanpur	–
Seto Basaune	Dev Raj Poudel	Begnas, Kaski	848
Seto Bose	Bishnu Prasad Tiwari	Begnas, Kaski	848
Seto Lamo	Hari Maya Poudel	Begnas, Kaski	848
Toriya	PGRU, ABD NARC	Garamani, Jhapa	–
Ujarka-B	Badri Prasad Kushwaha	Kachorwa, Bara	85
Ujarka-N	Nisahara Khatun	Kachorwa, Bara	85
Ujarka-R	Raj Kali Devi Gupta	Kachorwa, Bara	85

<sup>†</sup> Basmatiya did not germinate.<sup>‡</sup> PGRU, Plant Genetic Resources Unit..**Figure 1. Climatic parameters during growing season of sponge gourd in Malepatan, Pokhara.**

**Table 2. Evaluation descriptors for sponge gourd used in this study.**

Descriptor	Method
Days to emergence	Number of days from seeding to emergence. Recorded date of seeding and date of emergence to calculate
Days to first harvest for vegetable use	Number of days from seeding and first harvest for vegetable use
Days to last harvest for vegetable use	Number of days from seeding and last harvest for vegetable use
Harvesting period of fruit for vegetable use	Days to last harvest minus days to first harvest
Fruit skin thickness	Average of 3 fruits of different plants measured at central part of fruit during harvest for vegetable use
Flesh skin thickness	Average of 3 fruits of different plants measured at central part of fruit during harvest for vegetable use
Fruit length	Average of 3 fruits of different plants measured from blossom end to stem end during fruit harvest for vegetable use
Fruit perimeter	Average perimeter of 3 fruits of different plants measured in 3 parts (petiole end, centre and blossom end)
Fruit weight	Average weight of 3 fruits from different plants during harvest for vegetable use
Fruit number/plant	The number of fruits suitable for vegetable use during crop season: average of 3 plants
Fruit yield/plant	Weight of total fruits harvested for vegetable use: average of 3 plants

## Results and discussion

These landraces represent two ecoregions—mid-hills and *Terai* of Nepal. While *Terai* has a tropical climate, mid-hills have a subtropical to temperate climate. All these landraces except *Basaune* grew well and produced fruits. All climatic parameters were higher in the crop-growing period (Figure 1). During initiation of fruit harvest for vegetable purpose, minimum and maximum temperature, rainfall and relative humidity were high. This indicates that temperature is an important factor for tender fruit production. A cool night and warm day is favourable for higher yield and cool night, warm day and humidity induce female flowers (Davis 1996).

Multivariate analysis of variance indicated the overall significant test (Wilk's  $P < 0.001$ ) among these landraces. After obtaining a significant multivariate test we examined the univariate F test for each variable to interpret the respective effects. Analysis of variances were significant for first harvest days and fruit yield/plant, highly significant for last harvest days, fruit skin thickness, flesh thickness, fruit length, fruit perimeter, fruit weight and fruit number, but not significant for days to germinate (Table 3). *Ujarka-R* took the longest time to germinate and *Jhimni* germinated earliest. Standard deviation for days to germination was higher for Kachorwa population. This probably is due to high temperature in Kachorwa from where these landraces were collected whereas the trial was conducted in a lower-temperature site, Malepatan.

Tender fruits were harvested earliest from the Kachorwa population. Among these landraces, *Jhingani* produced fruits ready for harvest within the shortest period and *Lamo Bose* took the longest period to produce fruit for harvest. Standard deviation was the highest for days to first harvest in accessions from ABD collections because these accessions consisted of landraces collected from different locations. Earliest harvest may help to get high market value and to provide green vegetable during dry season. Many agronomical and indigenous practices can be used for better crop harvest. Transplants resulted in a better plant stand than the direct-seeded plants (Davis 1996). Delayed planting resulted in low first harvest yields. For the total harvest, yields from transplant were 1.2 times higher than those

Table 3. Phenological, fruit skin and flesh characteristics of sponge gourd landraces.

Landrace	Germination (d)		1st harvest (d)		Last harvest (d)		Fruit skin thickness (mm)		Flesh thickness (cm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Begnas, Kaski</i>										
Hariyo Chhoto	7.670b	1.16	106.3abcd	14.01	163.0ab	6.24	2.440cde	0.51	4.380bcde	0.54
Hariyo Basaune	8.000b	0.00	94.00abcde	7.00	156.3abc	11.50	3.000abcd	0.33	4.200bcde	0.29
Hariyo Lamo	7.000b	0.00	96.00abcde	11.36	153.3abcd	10.79	2.890abcde	0.19	4.010bcde	0.52
Lamo Bose	9.200ab	3.54	112.4a	1.41	150.2abcd	31.80	3.300abc	0.47	4.930b	0.87
Hariyo Bose	7.670b	1.16	90.30abcde	7.09	155.0abc	6.56	2.890abcde	0.84	3.910cde	0.40
Seto Bose	8.330b	0.58	105.7abcd	5.13	154.7abc	15.31	3.610a	0.10	4.470bcd	0.27
Seto Lamo	7.000b	0.00	103.7abcd	9.87	154.0abc	5.29	2.890abcde	0.54	3.820de	0.34
Seto Basaune	8.330b	0.58	106.7abcd	6.03	162.3ab	9.81	3.440ab	0.20	4.000bcde	0.22
Basaune	8.330b	0.58	97.30abcde	16.20	151.3abcd	5.77	2.500cde	0.50	4.430a	0.49
Mean	7.95	0.70	101.38	7.25	155.57	4.43	3.00	0.40	4.23	0.35
<i>ABD, Khumaltar</i>										
Jhingani	11.67ab	6.43	80.70e	10.60	148.3abcd	9.50	2.330de	0.58	3.470e	0.79
Toriya	7.000b	0.00	101.3abcde	5.69	169.3a	6.35	3.220abcd	0.19	3.700de	0.16
Jangali Ghiraula	11.67ab	5.51	111.3ab	19.10	165.0ab	7.00	2.670bcde	0.76	4.820bc	0.92
Jhimni	6.500b	0.00	104.5abcd	14.80	138.3cd	0.00	2.010e	0.00	4.570bcd	0.42
Sano Ghiraula	8.000b	0.71	103.0abcde	21.20	138.3cd	0.00	2.510cde	0.71	4.550bcd	0.04
Sagputti Ghiraula	11.67ab	6.43	85.70de	11.24	133.0d	10.39	3.060abcd	0.42	2.520f	0.60
Mean	9.42	2.51	97.75	11.88	148.70	15.19	2.63	0.45	3.94	0.88
<i>Kachorwa, Bara</i>										
Harihar Tagwa Bhadaiya	8.300b	0.71	103.1abcde	24.70	167.0a	2.83	2.450cde	0.12	4.340bcde	0.57
Ujarka-B	8.000b	0.00	87.30cde	10.97	149.3abcd	16.07	2.560bcde	0.20	4.230bcde	0.12
Lamka Ujarka	11.33ab	6.66	110.0abc	11.27	163.7ab	8.02	3.220abcd	0.19	3.730de	0.37
Hariharka	12.00ab	6.08	89.00bcde	10.82	150.3abcd	6.81	2.330de	0.34	4.590bcd	0.10
Ujarka-R	15.33a	6.35	92.00abcde	12.17	144.0bcd	12.29	3.280abc	0.68	4.620bcd	0.54
Ujarka-N	7.330b	0.58	96.70abcde	6.11	155.7abc	6.81	3.330abc	0.34	4.610bcd	0.41
Mean	10.38	3.08	96.35	8.80	155.00	8.90	2.86	0.46	4.35	0.35
P value	0.203		0.05		0.009		0.005		<0.001	
Kantipure			105							

SD, Standard deviation. Values followed by the same letter(s) are not significantly different at 0.05 level by DMRT. Kantipure was released variety (NARC 2000) and its traits were taken from VDD and SEAN (1995).

from direct-seeded plants (Davis 1996). Farmers hang some weight—usually a long chain of maize spadix—on the vine of sponge gourd for higher fruit yield and earlier fruiting and some farmers pinch the vine for the same purpose.

Tender fruits were harvested up to 169.3 days after seeding from *Toriya*. *Sagputti Ghiraula* had the shortest period from seeding to last harvest. The ABD population had high standard deviation for days to last harvest (Table 4). Periods of tender fruit harvest of all landraces are shown in Figure 2. *Toriya* had the longest fruit-harvesting period followed by *Hariyo Bose*. *Jhimni* had the shortest period of fruit harvest. Fruit-harvesting period is important for getting higher yield as well as for a regular supply of green vegetables. Farmers who grow very few plants in their home gardens prefer varieties having a long harvesting period. Relatively, Kachorwa landraces have a longer period of fruit harvest.

Farmers did not report fruit skin thickness as their selection criterion. However, fruit having thin skin is preferred for cooking purposes. Variation in fruit skin among these landraces indicates the possibility of selecting genotypes of desired skin thickness. Fruit skin thickness ranged from 2.01 to 3.61 mm. *Seto Bose* had the thickest and *Jhimni* the thinnest skin. Standard deviation of skin thickness was similar among the three collections, i.e. Begnas, Kachorwa and ABD. Flesh thickness is a more important trait than skin thickness. Means of flesh thickness were not significantly different among the Kachorwa population, while the other two populations had significantly different flesh thickness. Variation was higher in ABD collections. *Lamo Bose* had thickest flesh followed by *Jangali Ghiraula* and *Sagputti Ghiraula* had the thinnest flesh (Table 3).

Fruit characteristics and yield of sponge gourd landraces are given in Table 4. Fruit length and fruit number/plant have been considered important criteria for selection by sponge-gourd growers. Existence of highly significant differences in these traits indicates the huge variation being maintained by Kachorwa and Begnas farmers. ABD collections also represent unique genotypes. The Begnas population has longer fruits and the ABD collections shorter fruits. *Hariyo Lamo* had the longest fruit followed by *Hariyo Bose*. *Sano Ghiraula* and *Sagputti Ghiraula* produced the shortest fruits. Names of these landraces indicate the length of fruits, e.g. *Sano* means small and *Lamo* means long. The Begnas population has more diverse landraces for fruit length. Large genetic diversity was noticed among the landraces by Bajracharya *et al.* (1999). Landraces from Kachorwa were not significantly different for fruit length. Fruit characters are the most important descriptors used by farmers for distinguishing and naming the sponge gourd landraces (Bajracharya *et al.* 1999, Pandey *et al.* 2003; Yadav *et al.* 2003). Bajracharya *et al.* (1999) reported that farmers were consistent in identifying the landraces of sponge gourd but this was not true in all cases.

For fruit width, ABD collections had more variation. Begnas population has landraces having fruit of similar width as does the Kachorwa population. Perimeter of *Hariharka* was the highest and that of *Sano Ghiraula*, the lowest.

For commercial cultivation, fruit weight is an important trait. Diversity in fruit weight was higher in ABD collections followed by Begnas population. Begnas population had higher fruit weight than the other two populations. Heaviest fruit was produced by *Lamo Bose* followed by *Seto Basaune*. *Sano Ghiraula* and *Sagputti Ghiraula* produced the lightest fruits. Single fruit weight in these populations ranged from 49 to 334.3 g. Fruit weight was not significantly different among landraces of the Kachorwa population (Table 4). Fruit number is a relatively more important criterion for farmers. More variation in fruit number was found in ABD collections. This is due to different sources of collections. Fruit number was not significantly different among landraces of Begnas and Kachorwa populations. Number of fruits per plant ranged from 5 to 74. *Sano Ghiraula* produced the highest number of fruits per plant. *Sano Ghiraula* and *Sagputti Ghiraula* are the two landraces that have produced significantly higher number of fruits than others. Lowest fruit number was produced by *Harihar Tagwa Badaiya*. Landraces having longer fruit size produced lower number of fruits ( $r, 0.44^{**}$ ) (Table 5).





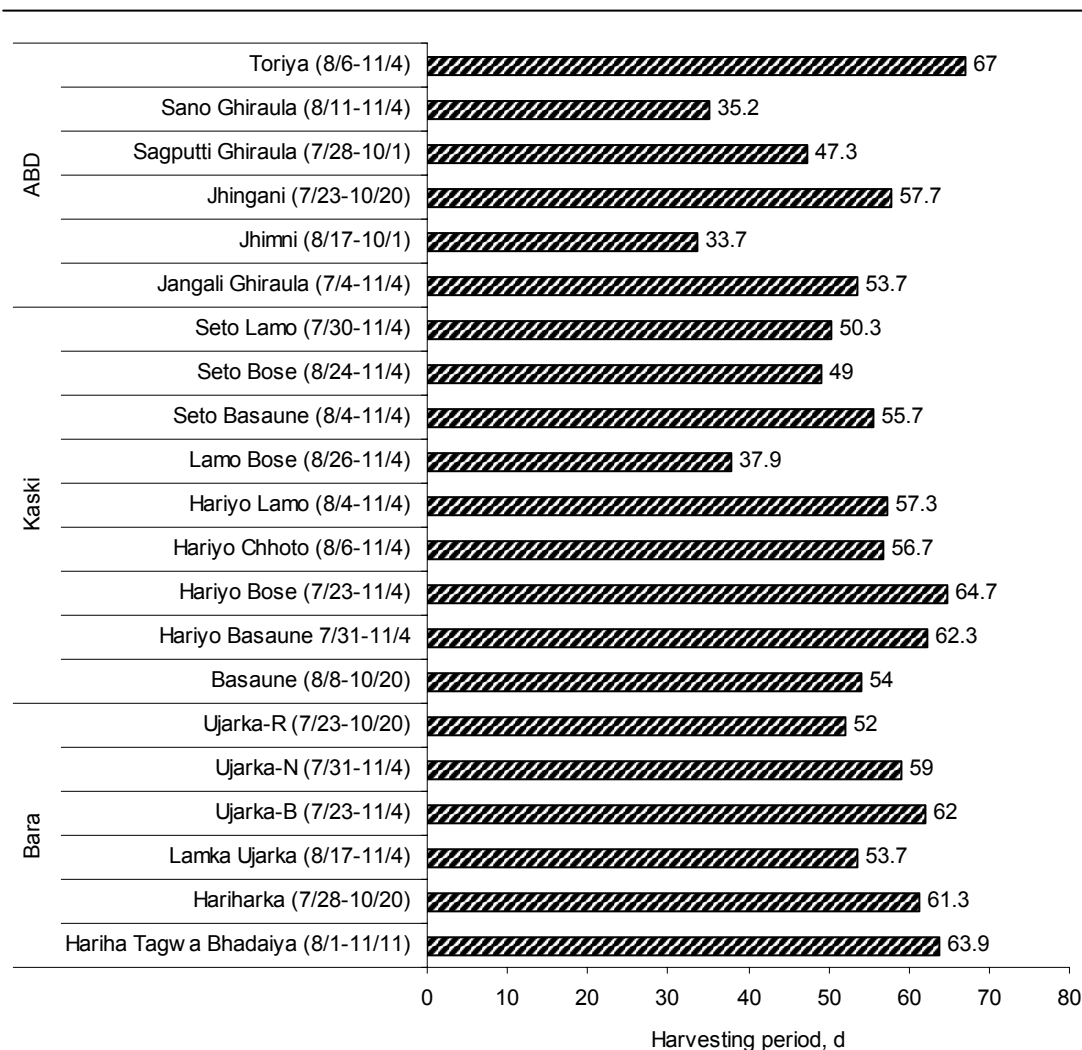


Figure 2. Fruit-harvesting period of different landraces of sponge gourd.

Table 5. Correlation coefficients among 11 traits measured from 21 landraces of sponge gourd.

Trait	1	2	3	4	5	6	7	8	9	10
Germination										
1 <sup>st</sup> harvest	−0.18									
Last harvest	0.01	0.20								
Harvesting period	0.15	−0.66**	0.61**							
Fruit length	−0.13	−0.03	0.22	0.20						
Fruit perimeter	0.05	0.01	0.36**	0.27*	0.31*					
Fruit weight	0.00	0.03	0.47**	0.34**	0.74**	0.51**				
Fruit/plant	0.01	−0.30*	−0.11	0.15	−0.47**	−0.22	−0.38**			
Fruit skin thickness	0.09	0.09	0.25	0.12	0.38**	0.46**	0.50**	0.04		
Flesh thickness	0.05	0.04	0.38**	0.26*	0.24	0.90**	0.52**	−0.22	0.43**	
Fruit yield /plant	−0.08	−0.23	0.335**	0.444**	−0.06	0.28*	0.11	0.52**	0.23	0.31*

\*, \*\* Significant at 0.05 and 0.01 level respectively.

Three landraces—*Hariharka*, *Basmatiya* and *Lamka Ujarka*—were characterized by Yadav *et al.* (2003). Variation was reported in *Lamka Ujarka* for leaf and node characteristics. Yadav *et al.* (2003) reported fresh fruit yield of 241.54 g and 166.14 g in *Lamka Ujarka* and *Hariharka*

respectively. *Chitkavra* had fresh fruit weight of 300 g in Kachorwa (Yadav *et al.* 2003). Pandey *et al.* (2003) have characterized five landraces (*Basaune*, *Hariyo Chhoto*, *Hariyo Lamo*, *Lamo Bose* and *Seto Lamo*) on-farm, of which *Hariyo Chhoto* is the most preferred one followed by *Hariyo Lamo* and *Basaune* in Begnas. *Seto Lamo* and *Basaune* were reported as rare landraces. Pandey *et al.* (2003) also reported both inter- and intralandrace diversity with most of the landraces having high productivity. *Hariyo Chhoto* is an early fruiting landrace with prolonged fruiting and remains tender for long period. *Hariyo Lamo* has long fruit with good yield. *Seto Chhoto* is less spongy and takes a short time to cook. However, the vines produce fewer fruits and set sponge early.

Farmers usually maintain 1–5 plants/household. Therefore, fruit yield/plant is more important than yield/hectare. On average, ABD collections produced greater fruit yield followed by the Begnas population. More variation in fruit yield also occurred in ABD collections (Table 4). Highest fruit yields were produced by *Toriya* followed by *Jangali Ghiraula* and *Seto Basaune*. *Lamo Bose* gave the lowest fruit yield. Significant fruit yield was not observed within the Kachorwa population. Among Kachorwa landraces, *Ujarka-N* produced the highest fruit yield/plant. The highest fruit yielder among Begnas landraces was *Seto Basaune*. A single plant of landraces can cover a large area if provided enough support. Farmers support vines on tall and large dead branches of trees. In some places a single plant can cover the complete roof area of a house. In such cases, these landraces may produce higher fruit yield than we have reported here. We supported vines with small dead guava branches. It is likely that due to this restriction on expansion of vines, these landraces may not have yielded their full potential.

Correlation coefficients among these 11 characters are given in Table 5. These are useful, especially for selection of genotypes. Negatively associated traits were fruit/plant with days to first harvest, fruit/plant with fruit length, harvesting period with days to first harvest, and fruit/plant with fruit weight. Highest character correlations were in fruit characters (Cruz *et al.* 1997). The *L. acutangula* group has lower overall variation and character means compared with *L. cylindrica* (Cruz *et al.* 1997). For increasing fruit number we can consider fruit length and fruit weight as primary traits. Similarly for increasing fruit yield/plant, the primary traits were days to last harvest, harvesting period and fruit/plant. The second important group of traits was flesh thickness and fruit width. Simply by understanding these associations among traits, farmers can impose selection intensity for genetic advance. For example, a landrace with a long harvesting period could, if selected, produce higher fruit yield.

## **Conclusion**

There were significant differences among the landraces diversity studied for farmers' preferred traits, e.g. tender fruit yield, fruit weight and fruit harvesting period. Because of diverse needs of farmers in diverse environments, different landraces are being maintained. Options for improving these landraces exist by reshuffling promising attributes. Landrace improvement is another option for better crop harvest. Improvement work should be initiated on farmer and consumer traits. The correlation study indicated that fruit length and fruit weight are important traits for increasing tender fruits. A strategy of maintaining seeds by individual farmers should be considered for conserving landrace diversity on-farm.

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## Traditional seed supply system of sponge gourd in mid-hills and plains of Nepal

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### Abstract

The traditional seed supply system of sponge gourd is the least understood in Nepal. So a modified Focus Group Discussion (FGD) was conducted in Kaski and Bara sites to find the traditional methods and models of sponge gourd seed supply system covering more than 23% of households (HHs). The finding of this study was that the main source of seed was farmer's own retention. Receiving seed from others as a gift was a prominent method of seed acquisition. In both sites, farmers mainly followed fruit selection based on certain fruit quality traits for retaining the seeds. Farmers do not select sponge gourd plants for seed production. In both sites, farmers store the dried fruits in safe places and hang them in the house and extract the seeds from the fruits just before planting in their fields. Fruits free from diseases and insects along with better appearance, straight shape, big size and long size are the main criteria of fruit selection for seed. Seeds that are plump and bold, and seeds from the blossom-end of fruit, are the main basis of seed selection. Sponge gourd green fruits are mainly used as a vegetable and the seeds are used for oil extraction for medicinal uses; the sponge is used for cleaning. In Bara, sponge gourd seed has entered into the formal seed system, having the potential to cover a large area in a short time. Both sites are rich in sponge gourd varietal diversity, but there is a threat of losing this resource in Bara. There is a need to address farmers' aspirations about sponge gourd. Seed production and distribution of endangered varieties are recommended.

**Key words:** Fruit, seed flow, seed production, seed system, selection criteria, sponge gourd

### Introduction

Sponge gourd (*Luffa cylindrical* (L.) Roem) is one of the popular summer and traditional vegetable crops in Nepal. This crop has been domesticated in Nepal since time immemorial. Indo-Burma is reported to be the centre of diversity for sponge gourd (Joshi *et al.* 2004). Nepal is rich in sponge gourd diversity, so it was one of the target crops identified by the global project "Strengthening the scientific basis of *in situ* conservation of agricultural biodiversity" in Nepal. Sponge gourd is a cross-pollinated crop and monoecious plant and diclinous flower, where male and female flowers are located in different parts of the same plant. It is commonly grown in home gardens and in large areas in certain hill and plain areas of Nepal. The fruits of sponge gourd contain a fibrous vascular system, which is consumed as a fresh vegetable and the sponge of matured fruit is used for cleaning (Pandey *et al.* 2003). The cultivation of sponge gourd is easy, and it is used as an income-generating commodity. In Nepal, it is grown in living trees, dead branches, wall, roofs, etc. (Joshi *et al.* 2004). The private and public sectors dealing with seeds of different crops are very poor, even for the major food and vegetable crops. So, except for a few private parties, no organization is dealing with the seed system of sponge gourd. Each year farmers decide how much seed to plant and determine where that seed came from (Baniya *et al.* 1999). Bellon (1996) clarified the concepts of seed flows, variety choice, seed selection and seed management. Attempts have been made to study the seed supply system of sponge gourd in Kaski (hill) site and Bara (plain) site of the *in situ* global project. Yadav *et al.* (2003) suggested studying the seed system of sponge gourd to understand the maintenance of its seed. The seed supply system of sponge gourd is not well understood and this study was necessary to develop a strategy for its conservation and proper utilization.

## Materials and methods

The modified Focus Group Discussion (FGD) was applied to collect information and knowledge about the sponge gourd seed system in Kaski and Bara ecosites. To capture about 20% of the households (HH) from each project site, the 22 previously established farmer groups in each site were used. The survey method followed in this study was as follows.

A structured questionnaire with 20 questions about the seed supply system of sponge gourd was prepared. From each group 5–6 farmers knowledgeable about sponge gourd, farmers representing their HH were randomly selected. Every one of them was asked to select one other nearest neighbour or friend whom he/she knew was knowledgeable about the sponge gourd seed system. Thus in each group physically there were 5 or 6 persons and another 5 or 6 of their friends who were not present in the discussion. After the general information was collected from the group, each farmer was asked about sponge gourd seed and when the information of the 5 or 6 farmers was recorded from each question in the group, then each farmer was asked the same question to answer on behalf of his/her friend. The total HHs, total groups, HH/farmers surveyed with the representation of male and female farmers is presented in Table 1.

**Table 1. Short description of the site and farmers/HH surveyed in the two sites, 2004.**

Description	Begnas, Kaski	Kachorwa, Bara
Total HHs	941	914
Total group, no.	22	22
Total farmers/HH survey	245	217
a. Male farmers, no.	131	201
b. Female farmers, no.	114	16
Altitude range, masl	600–1400	80–90
Average annual rainfall, mm	3979	1515

Source: Baseline survey reports of Begnas and Kachorwa.

Information and knowledge about the sponge gourd seed supply system were collected and compiled manually. The assumption of following this modified FGD was that the information and knowledge about this subject is limited and not much divergent from the rice seed supply system. The enumerators visited the actual place of the group, collected the farmers at one place, discussed with them in the group of 5 or 6 and completed the questionnaire. A total of 245 and 217 farmers from 22 groups in each site from Kaski and Bara participated in the discussion respectively (Table 1).

## Results and discussion

### Seed flows

The source of seed, its acquisition and replacement are different in both sites. The majority of farmers retain their own sponge gourd seed in Kaski (78%) and Bara (55%) (Table 2). Similarly 19% of farmers from Kaski and 32% from Bara received seed from their neighbours. About 3% of Kaski farmers received seed from development organizations (extension, research, NGOs, CBOs, etc.), whereas in Bara, farmers took seed from their relatives. One major deviation in Bara is that about 10% of farmers bought sponge gourd seed from market (Table 2).

**Table 2. Source of sponge gourd seed, 2004.**

Source	Kaski			Bara	
	No.	Percent	Remark	No.	Percent
Own retention	190	78		119	55
Neighbour	46	19		70	32
Relative	—	—		7	3
Market	—	—		21	10
Other (Development organizations)	9	3		—	—
Total	245	100		217	100

Based on the other sources of seed (55 Kaski farmers and 98 Bara farmers), the acquisition of sponge gourd was calculated and is presented in Table 3. In Bara, about 33% of farmers exchanged sponge gourd seed for the seed of sponge gourd and/or other crops from their neighbours. In Kaski, about 84% of farmers received seed from their relatives and neighbours as a gift and the remaining farmers received sponge gourd seed free from a development organization, i.e. mainly from the *in situ* project and agriculture development office. The project staff distributed mainly the seed of *Basaune Ghiraula* (local variety of that location) as the diversity kits in Kaski. Similarly, about 46% of Bara farmers receive seed from their relatives and neighbour as a gift and about 21% of farmers even bought sponge gourd seed from the market (Table 3). In Bara, the farmers practise seed exchange and buy sponge gourd seed from market. So, sponge gourd seed is one of the business commodities in Bara and even hybrid sponge gourd seed was brought there, but in Kaski sponge gourd seed has not entered the formal marketing system (Table 3).

**Table 3. Basis of acquisition of sponge gourd seed, 2004.**

Basis	Kaski		Bara	
	No.	Percent	No.	Percent
Exchange	—	—	32	33
Gift	46	84	45	46
Purchase	—	—	21	21
Free	9	16	—	—
Other	—	—	—	—
Total	55	100	98	100

In Kaski, there was no regular seed replacement system. But, if their varieties did not perform well, were damaged, spoiled or lost, and some varieties were doing well in other fields, the farmers managed to obtain the seed of these particular varieties. This was very common practice in Kaski where all farmers did this. Similarly, in Bara if their varieties do not perform well or better varieties are observed in other field or better varieties are available in the market, the farmers replace their old varieties on a regular basis (Table 4). The majority of Bara farmers replaced their sponge gourd seed after only a 3-year period (Table 4).

**Table 4. Sponge gourd seed replacement in Bara, 2004.**

Period	No.	Percent
Every year	4	2
Every 2–3 year	61	30
More than 3 year	103	50
No response	36	18
Total	204	

### Seed production

When the farmers were asked whether they fix plants or fruits for seed production in Kaski, 88% of 245 farmers yes, and in Bara 55% of 217 farmers said they practised fixing plants/fruits for seed production. However, very few farmers select/fix the plants and the majority of farmers focused mainly on fruit criteria. A few Kaski farmers selected plants based on their fruiting character, flower colour and diseases as well as resistance to insect attack. In Bara, not a single farmer selected plants for seed production. Pandey *et al.* (2003) found that fruit characteristics are the most important descriptors used by farmers for distinguishing and naming the sponge gourd varieties. Farmers of both sites did not follow additional operations for seed production in plants/fruits, except regular supervision of the selected fruits and some farmers just market the fruits selected for seed purpose. Farmers were not aware about the cross-pollinating nature of sponge gourd; and they were not following any measures to control outcrossing for producing genetically pure seed.

The farmers of both sites extensively followed fruit selection at different times by fixing certain set criteria. In Kaski 228 (93%) farmers and in Bara 200 (92%) farmers followed fruit/seed selection practices. About 87% of farmers select the fruit before harvesting in Kaski and in Bara 54% of farmers select the fruits during the harvesting time (Table 5). About 10–12% of farmers selected fruits after harvest also in Bara and Kaski respectively (Table 5).

**Table 5. Time of fruit selection, 2004.**

Time	Kaski		Bara	
	No.	Percent	No.	Percent
Before harvest	199	87	72	36
During harvest	—	—	108	54
After harvest	26	12	20	10
No response	3	1	—	—
Total	228	100	200	100

Farmers followed different ways of selecting the fruits from any particular plant. Most selected the better fruits from any plant (Kaski farmers, 84% and Bara farmers, 67%). Only 8% of farmers looked for better plants in both sites (Table 6). Some farmers did not select either plant or fruit for seed production, but kept any fruits from any plant for seed purpose.

**Table 6. Method of sponge gourd selection, 2004 (multiple response).**

Method	Kaski		Bara	
	No.	Percent	No.	Percent
Better fruits from better plants	19	8	17	8
Better fruits from any plant	192	84	146	67
Any fruit from any plant	34	15	26	12
Other (first fruit from any plant)	—	—	28	13
Total (n)	228	100 (7)		100

Farmers are very much concerned about quality of fruit for seed production. They looked for the better morphological and agronomical characteristics of the fruits for seed purposes. Kaski farmers selected sponge gourd fruits that were straight (60%), free from diseases and insects (55%), better looking (51%), soft or *Bose* type (44%), true to type (43%), big and long (33%) and first beard fruit (27%), whereas Bara farmers selected fruits that were free from diseases and insects (63%), better looking (54%), straight (50%), big and long (48%), first beard fruit (17%) and fully matured fruit (15%). Some farmers from Kaski preferred third or fourth beard fruit and fruits from sunny places (Table 7).



**Table 7. Sponge gourd fruit selection criteria, 2004 (multiple response).**

Criterion	Kaski		Bara	
	No.	Percent	No.	Percent
Straight fruit	136	60	100	50
Free from diseases and insects	126	55	126	63
Better looking fruit	116	51	108	54
Soft (Bose type)	99	44	—	—
True to type (pure)	97	43	—	—
Big and long (slender) fruit	76	33	96	48
First beard fruit	61	27	34	17
Fully matured fruits	8	4	30	15
High yielder	—	—	14	7
Other (3 <sup>rd</sup> or 4 <sup>th</sup> fruit, from sunny places)	14	6	—	—
Total (n)	228	100(223)	200	100(154)

Note: Most of the farmers use more than one selection criterion, so each criterion is treated as independent and percentages are calculated accordingly.

Farmers of both sites have fixed sponge gourd seed selection criteria and follow seed selection procedures. Most farmers select bold, plump/fully filled seed. Kaski farmers prefer seed from the blossom-end of the fruit, as they believe that these seeds would bear big fruits after planting. But 1% of Bara farmers thought that seed from the stem end (peduncle side) would be better (Table 8).

**Table 8. Sponge gourd seed selection criteria, 2004 (multiple response).**

Criteria	Kaski			Bara		
	No.	Percent	Remark	No.	Percent	Remark
Plump, bold, fully filled	193	85		149	75	
Seed from the blossom end	117	51	Fruits will be bigger	—	—	
Fruits with shining surface seed coat	37	16		—	—	
Big (thick)	—	—		27	14	
Uniform size and good shape	—	—		24	12	
Seeds from stem end (peduncle side)	—	—		2	1	Base of fruit
Total (n)	228	100 (52)		200	100 (2)	

Note: Most of the farmers put more than one selection criteria, so each criterion is treated as independent and percentages are calculated accordingly.

### Seed extraction

There is not much variation in time and method of sponge gourd seed extraction from the fruits. Over 93% of farmers of both sites extracted seeds from the fruits just before planting (Table 9), which indicated that farmers only have to take care of fruit. This is because the seeds remain safe inside the fruit and are easy to store. About 2 and 7% of farmers extracted seeds any time between harvesting and planting of seed in Kaski and Bara, respectively. Only 1% of Kaski farmers extracted seed immediately after harvesting the fruit (Table 9), which needs to be verified.

**Table 9. Time of sponge gourd seed extraction from fruit, 2004.**

Time	Kaski		Bara	
	No.	Percent	No.	Percent
Immediately after fruit harvest	2	1	—	—
Just before planting	221	97	185	93
Anytime between harvest and planting	5	2	15	7
Total	228	100	200	100

The majority of Kaski farmers break the fruit blossom end and take the seed out; very few farmers cut the fruit at the middle of the fruit and extract the fruit. In most cases, well-dried fruits are stored in dry and shady places and seeds are extracted from the fruits, which are planted immediately in Kaski. In Bara also, farmers cut the blossom end of the dried fruits just before planting and plant the seed. Very few farmers cut the stem end (peduncle side) of the fruit and take seed.

### Fruits and seed storage

Many farmers harvest the matured fruits and store them in safe places. Generally the farmers harvest the fruits when the plants die and dry fully. Then the fruits are hung by ropes from the ceiling or roof. Almost all Kaski and Bara farmers store the sponge gourd fruits in this way (Table 10). In sponge gourd, if the fruits selected for seed purpose are in safe places, the farmers preferred to keep the selected fruits in the plant itself until cleaning the land for next season crop planting; and dry fruits are used mainly for seed purposes. In Bara, 10% of farmers stored the fruits inside *almari* or big baskets (*Bhakari*) in the house. Similarly, some farmers at both sites store the fruits in a dry place, away from the reach of children and mice (Table 10).

**Table 10. Sponge gourd fruits storage method, 2004 (multiple response).**

Method	Kaski		Bara	
	No.	Percent	No.	Percent
Harvest fruits after plants die and hang up by rope	220	97	196	98
Dry the fruit sufficiently and store	10	4	—	—
Store the seed in dry and away from mice/children	50	22	20	10
Store inside Almari/Bhakari	—	—	20	10
No response	(52)	—	(38)	—
Total (n)	228	100 (23)	200	100 (18)

Note: Many farmers followed more than one method to store the fruits, so each method is treated as independent and percentage is calculated accordingly.

The majority of farmers hang the fruits on the ceiling of the balcony (*Baranda (Dalan), pali*) and even the house wall. About 93% of Kaski farmers and 76% of Bara farmers store fruit by hanging (Table 11). The storage place can be inside or outside the house.

**Table 11. Sponge gourd fruit storage places, 2004 (multiple response).**

Place	Kaski		Bara	
	No.	Percent	No.	Percent
Hang/place on the ceiling of roof, balcony, baranda, wall	212	93	155	78
Inside or outside house	52	23	20	10
<i>In situ</i> (leave in the plant, in accessible roof or tree)	13	6	3	1
No response	(49)	—	22	11
Total	228	100 (22)	200	100

Note: Many farmers stored the seed in more than one place, so each place is treated as independent and percentages are calculated accordingly.

Mostly seeds remain inside the fruit itself. A few Kaski and Bara farmers extract sponge gourd seeds before planting (Table 9). These farmers generally keep the seed in cloth or cloth bags or plastic bags and store them in some sort of container like an earthenware pot, wooden box (*Sandua*). Sometimes, if the fruits are broken or destroyed by mice, seeds are taken out, packed in the cloth or plastic bags and stored in a safe place.

### Uses of sponge gourd

In both the sites, sponge gourd fruits are exclusively used as a vegetable for home consumption and a few farmers even sell the fruits. In Kaski, farmers use seed only for planting, but Bara farmers had other uses for seed and fruits as follows:

- Seed oil applied on head provides coolness
- Seed oil is used for medicinal purposes
- Green fruits are applied on fresh wounds for coolness and healing
- The sponge from the fruit is used to wash the body and clean utensils.

All together, 16 sponge gourd varieties in Kaski and 13 varieties in Bara were reported. *Basaune*, *Hariyo Chhoto*, *Hariyo Lamo* and *Seto Ghiraula* were very popular varieties in Kaski whereas *Galphulia*, *Basmatiya*, *Taguwa* and *Hariharkla Lamka* were commonly grown in Bara. Khatiwada *et al.* 1999, Pandey *et al.* 2003 and Yadav *et al.* 2003 also reported different landraces of sponge gourd in Kaski and Bara. In Bara, sponge gourd seeds are available in the street (Bhadaiya, Dehati, SPL long white 32, Nema sweta and Long white) and in local AGRO Vets (Java F1, White seeded and SPL long white 32). The sources of all varieties were India, and JavaF1 and White seeded are hybrid varieties. The names of the reported sponge gourd varieties are provided in Box 1, ranked according to their popularity (first being the most popular).

#### Box 1. List of sponge gourd varieties reported in 2004.

##### Kaski

- |                     |                      |                      |
|---------------------|----------------------|----------------------|
| 1. Basaune Ghiraula | 6. Seto Begnase Lamo | 11. Hariyo           |
| 2. Hariyo Chhoto    | 7. Lamo Kalo         | 12. <i>Lamo</i>      |
| 3. Hariyo Lamo      | 8. Seto Chhoto       | 13. Tirahi           |
| 4. Seto Ghiraula    | 9. Chhoto Bose       | 14. Hariyo Majhauila |
| 5. Seto             | 10. Seto Lamo        | 15. Tito Ghiraula    |

##### Bara

- |                   |                       |                     |
|-------------------|-----------------------|---------------------|
| 1. Galphulia      | 6. Lamkaujjarka       | 11. Kaliyaka/Kariya |
| 2. Basmatiya      | 7. Galphuliaujarka    | 12. Hybrid          |
| 3. Taguwa (Tagwa) | 8. Galphuliahariyarka | 13. Tagawahariyarka |
| 4. Lamkahariyarka | 9. Tagawa ujarka      |                     |
| 5. Raila          | 10. Ghiuwala          |                     |

Yadav *et al.* 2003 reported that Chitkavra sponge gourd variety is rare, and during this survey it was not reported, so it might have already lost from the site. *Ujjarka* means whitish, *Hariharka* means green and *Kariya* means darkish in Bhojpuri language.

### Farmers' opinions

During the survey, farmers were asked their opinions about sponge gourd in both the sites. Their opinions are summarized in Box 2. Farmers from both sites are looking for better varieties and new technology for sponge gourd cultivation.

**Box 2. Farmers' expectation/opinions about sponge gourd, 2004.****Kaski**

Need for high-yielding, tasty, aromatic, soft, long fruit type varieties  
 Need for new technology  
 Need for marketing  
 Treatment for stem-cutting insects

**Bara**

Need for aromatic, high-yielding, early maturing, long fruit type varieties  
 Need for technology and technical advice  
 Need quality seed  
 Need for plant protection measures

**Conclusion**

Sponge gourd is the important vegetable crop in Nepal and rich in varietal diversity. The study of sponge gourd seed supply system was not available from any source. Both Kaski and Bara have more or less the same type of traditional sponge gourd seed supply system. In Bara, seeds of hybrid sponge gourd were available in the market and they are under cultivation. The hybrids were high-yielding, bear big fruits and are a short plant type. So, there is a possibility of losing sponge gourd landraces from Bara, which needs careful attention to conserve the available landraces. The farmers of both sites have high expectations in terms of technological support and services for sponge gourd cultivation. In particular, NARC should be proactive in conserving and using the sponge gourd germplasm for varietal development in future. The modified approach of FGD is very effective to collect information in a short period of time, but its accuracy may be low owing to its methodological deviation; thus, the findings of this study should be used with care.

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## Cucumber seed supply system in Kaski and Bara districts of Nepal

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### Abstract

A modified Focus Group Discussion (FGD) was conducted in Kaski and Bara sites to find out farmers' methods and models of the cucumber seed supply system covering about 20% of households (HHs) in Kaski and about 9% of HHs in Bara. Farmer-saved seed was the main source in Kaski but market was the prominent source of cucumber seed in Bara. As only one surveyed farmer grows cucumber for seed purposes in Bara, mostly the findings from the Kaski study are reported here. In Kaski, farmers replace the seed when their varieties fail and better varieties are available. They select the better fruits from any standing plant and extract the seed after a few days of harvesting fruits. Big size fruit, fruits free from disease and insect damage, fruits that are tasty, better looking and straight are the main selection criteria for seed from fruit. Traditionally the wet seeds of cucumber are placed in an inner wall of the house until planting time. Some farmers pack the dry seed in cloth or plastic bags and store in containers in safe places. Fruits are mainly used as fresh vegetable and pickle, and seeds of cucumber can be used for different purposes. Kaski is rich in cucumber varietal diversity, but there is a possibility of losing cucumber landraces from Bara. Seed production and strengthening seed supply system through distribution of endangered landraces are recommended.

**Key words:** Cucumber, fruit, seed flow, seed production, seed selection criteria

### Introduction

Cucumber (*Cucumis sativus* L.) is a cross-pollinated crop and monoecious plant with diclinous flower, where male and female flowers are located in different parts of the same plant. Cucumber is a common fresh vegetable grown in many parts of Nepal. Although it is mainly used fresh, it has multiple uses. Nepalese farmers have grown cucumber for generations in diverse domains during summer and rainy seasons. Generally cucumber is grown in a small area in home gardens, and is grown as a commercial crop in different parts of Nepal and sold in the market all year around as an important vegetable crop. The centre of diversity of cucumber is India (Simmonds 1979). Nepal also is very rich in cucumber varietal diversity. This is why cucumber was one of the target crops identified by the global project "Strengthening the scientific basis of *in situ* conservation of agricultural biodiversity" in Nepal.

In Nepal the private and public sectors dealing with seeds of different crops are very poor and do not deal much with seeds of local varieties of vegetable crops like cucumber. The cucumber seed supply system is poorly understood in Nepal. Therefore, an understanding of this seed system is very important to develop a strategy for conservation and proper utilization of this crop. Seed production, seed selection, seed flow, seed storage and other practices followed by farmers have an impact on genetic diversity (Baniya *et al.* 2003). Bellon (1996) clarified the concept of seed flows, varietal choice and seed selection and seed management. So, attempts have been made to study the cucumber seed supply system in Kaski (hill site) and Bara (plain site) of the *in situ* global project.

### Materials and methods

To study the cucumber seed supply system in hills and plain of Nepal, the modified Focus Group Method (FMG) was applied in Kaski and Bara ecosites of the *in situ* global project. To capture about 20% of HHs from the each project site, the previously established 22 farmers' groups in each site were used. The methodology was discussed in one of the National Multidisciplinary Group (NMDG) meetings and agreed to follow FMG for this survey. The survey method followed in this study was as follows.

A structured questionnaire with 20 questions about the seed supply system of cucumber was prepared. From each group 5–6 farmers having knowledge of the cucumber seed system and representing the areas were randomly selected. Each was requested to select one other nearest neighbour or friend whom he/she knew to be knowledgeable about the cucumber seed system. Thus in each group physically there was 5 or 6 persons and other 5 or 6 of their friends who were not present in the discussions. After discussing in the group for the general information, each farmer was asked about the cucumber seed supply system and when the information of the 5 or 6 farmers was recorded from each question, then each farmer was asked the same question to answer on behalf of his/her friend for the same question. The total HHs, total group, HHs surveyed with the representation of males and females is presented in Table 1.

**Table 1. Short description of the ecosite and farmers/HH surveyed in the two sites, 2004.**

<b>Description</b>	<b>Begnas, Kaski</b>	<b>Kachorwa, Bara</b>
Total HH	941	914
Total group	22	22
Total farmers/HH surveyed	245	77
a. Male, no.	131	67
b. Femal, no.	114	10
Altitude range, masl	600–1400	80–90
Average annual rainfall, mm	3979	1515

Source: Baseline surveys of Begnas and Kachorwa, 2000.

Information and knowledge about cucumber seed supply system were collected and compiled manually. The assumptions of following this modified FGD were that the information and knowledge about this subject are limited and not much different from the rice seed supply system. The enumerators visited the actual place of the group, collected the farmers at one place, discussed with them in the group of 5 or 6 and filled out the questionnaire. All the participating 245 farmers from 22 groups grow cucumber in Kaski. About 47% of participants were female. In Bara, out of 217 participating farmers 77 grow cucumber and only 13% of them were female participants. Out of 22 groups, cucumber was cultivated only by 9 groups in Bara.

## **Results and discussion**

### **Seed flows**

The source of seed, its acquisition and seed replacement system are different in many respects at both sites. In Kaski, about 74% of farmers retain their own cucumber seed for next season planting. The seed received from development organizations (extension, research, NGOs, CBOs, etc.) was 12%. About 5% of farmers also purchased seed from the market (Table 2). In Bara, the source of seed is the market and only one farmer retains cucumber seed for his purposes (Table 2).

In Kaski, out of 245 farmers, only 63 acquired cucumber seed from other sources, e.g. seed from development organizations, which was free and its share is 12%; the neighbours provide cucumber seed to their friends as a gift in Kaski. There is a system of cucumber seed buy-and-sell (5%) in Kaski. In Bara, farmers purchased cucumber seed from local street seed sellers and AGRO vets. Seed of all but one variety was imported from India and the seed sellers also were from India.

**Table 2. Sources of cucumber seed, 2004.**

Source	Kaski			Bara		
	No.	Percent	Acquisition	No.	Percent	Acquisition
Own retention	182	74	Saved	1	1	Saved
Neighbour	23	9	Gift	–	–	–
Relative	–	–	–	–	–	–
Market	12	5	Purchase	76	99	Purchase
Other (development organization)	28	12	Free	–	–	–
Total	245	100	–	77	100	–

In Kaski, there was no regular interval of cucumber seed replacement. If the fruits are deformed, deteriorated plants are diseased, dead or did not perform well, farmers change the seed any time. When farmers observed better-performing varieties in others' fields, they managed to obtain the seed from their neighbour, relative or other villages. But in Bara, the farmers totally depend on market for cucumber seed, so they all changed the seed every year.

### Seed production

When farmers were asked whether they fix/select plants/fruits for seed production, 223 (91%) farmers in Kaski said they followed this process but all practised selection based on the fruit only, not the plant. Pandey *et al.* (2003) reported that fruit characteristics are important morphological traits that matched with the meaning of farmer-named cultivars in sponge gourd. In Bara, only one farmer out of 77 followed fixing of plants/fruits for seed selection purpose. They also never fix the plant, only the fruit. For seed production in cucurbits, farmers focused mainly on fruit. In Kaski, when selecting fruits for seed production about 50% of farmers give the fruits regular care, 42% of farmers provide better support for the fruits and the remaining farmers cover the selected fruits to protecting from theft by children and other people. Farmers were not aware of the outcrossing nature of cucumber and no precautions were taken to produce pure seeds.

In reply to other questions about the fruit and seed selection process, 76% of farmers in Kaski followed this selection procedure, but in Bara only one farmer practised fruit and seed selection operations. So, in the following discussion only the Kaski information is considered unless mentioned. About 96% of farmers select cucumber fruits before harvesting for seed purposes (Table 3) and almost 81% of farmers select better fruits from any plant. A few farmers look for better fruits from better plants.

**Table 3. Time and methods of fruit selection, Kaski, 2004.**

Time	No.	Percent	Method	No.	Percent
Before harvest	179	96	Better fruits from better plants	5	3
During harvest	–	–	Better fruits from any plants	152	81
After harvest	8	4	Any fruit from any plant	30	16
Total	187	100	Total	187	100

So, farmers select better fruits from any plant before harvest and store for seed purpose. This is true for Bara also. Fruits that are big and high-yielding (71%), free from diseases and insects (67%), tasty (62%), first bread fruit (57%), better looking (55%) and straight fruit (50%) were the main criteria for cucumber fruit selection. Similarly, true to type, early-matured fruits, long sized fruit, fully matured and soft in eating are the other important criteria of fruit selection (Table 4).



The seed selection criteria for cucumber seed were plumpness (true for Bara also) bold size, fully filled and better looking. Almost 75% of farmers extracted the seed a few days after fruits were harvested and about 22% of farmers extracted the seed immediately after harvest. It is difficult to save the selected cucumber fruits from theft, damage by mice and rotting in the storage, so farmers tend to extract the seeds as early as possible after harvest and store the seed in the places. More than 81% of farmers extracted the seeds from the fruits, did not wash the seeds, dried and stored the seeds safely. The rest (19%) extracted the seeds, washed them with water, and stored the seeds in safe places. In Kaski, some farmers think that if the seeds are washed, the fruit produced by these seeds will be tasteless. In Bara, farmers extract the seed, sun dry it, mix it with ash, pack it in cloth or plastic, put it into containers and store seed inside the house.

**Table 4. Cucumber fruit selection criteria, Kaski, 2004 (multiple response).**

Criteria	No.	Percent	Remark
Big fruit and high yielding	132	71	True for Bara
Free from diseases and insects	126	67	True for Bara
Tasty (good taste)	115	62	
First beard fruit	107	57	
Better looking	103	55	True for Bara
Straight fruit	94	50	
True to type (pure)	76	41	
Early maturing type	48	26	
Long fruit	23	12	True for Bara
Fully matured fruit	18	10	
Soft in eating	15	8	
Other (3 <sup>rd</sup> or 4 <sup>th</sup> beard fruit, saved from theft)	13	7	
Total	187	100 (366)	

Note: Most of the farmers put more than one selection criteria, so each criterion is treated as independent and percentage are calculated accordingly.

### Fruit and seed storage

About 40% of farmers conserve the fruits by keeping them in an upright position either in the window or inside the house. Some farmers hang the fruits in the balcony (*Siko*) and a few cover the fruits with a netted bamboo mat (*Doko*). In Bara, the farmer ties the fruits with rope and hangs them on the wall for 3–4 days before extracting the seed. Because of the fear of loss of the fruits, about 65% of farmers keep fruits inside their house and some (20%) hang the fruits inside their balcony. Very few farmers even store the cucumber fruits buried in rice or finger millet grains inside *Bhakari*. Generally, the fruits and seeds are stored in dark, dry, cold places, out of reach of mice and children.

The seed storage methods of cucumber are different and very traditional. Out of 187 seed-producing farmers, 182 extract the wet seeds from the fruits; with its natural sticking capacity the seeds are put in the house wall without washing, and remain there until the planting season without much loss and deterioration of the seed (Table 5). The other similar practice of seed storages is to apply cattle dung to the wall and the wet cucumber seed is put on it for storage. About 32% of farmers followed this method. The other practices of seed storage are drying the seed, packing it in cloth or plastic bags and storing the pack inside locally made containers, bottles, small earthenware pots or wooden boxes (*Sandus*) to protect seeds from mice.

While storing on the wall, some farmers put the seed behind the wall of the window or door and some put the packed seed in the wall hole (*Khopa*). The common containers used in Bara for seed storage are small earthenware pots or plastic pots. In Kaski, 187 farmers completed cucumber seed production steps but only 182 were able to retain seeds finally.

**Table 5. Cucumber seed storage methods, Kaski, 2004 (multiple response).**

Method	No.	Percent
Newly extracted wet seeds put on the wall without washing seed	182	97
Put cow dung on the wall and put the wet seed on it	59	32
Dry seed, pack inside cloth and put inside container	47	25
Pack inside cloth and hang on ceiling	7	4
Put inside plastic bag and store	2	1
Total (n)	187	100 (59)

Note: Most farmers used more than one method, so each method is treated as independent and percentages are calculated accordingly.

### Uses of cucumber

The main use of cucumber is as a fresh vegetable (83%), the second common use is fermented pickle using ripened fruits (74%) (Table 6). The use of green or ripened cucumbers is more common during festivals such as *Dashain* and *Tihar*. Some farmers perceived that cucumber fruit juice and its inner soft portion are good during hot days

**Table 6. Different uses of cucumber fruits and seed, Kaski and Bara, 2004 (multiple response).**

Use	No.	Percent
Fresh/green fruits used as fresh vegetable	205	83
Pickle from ripened green fruits	183	74
Seed used for fever problem	134	55
Fresh/Green vegetable	168	67
Ripened fruit used as such	24	5
Fruit water used for gastric and stomach pain	Some	—
Seed oil is used for massage in painful areas	Some	—
Cucumber seed after removing seed coat is put in <i>Bunia</i> (local food)	Some	—
Fruit juice and inner soft parts are good during hot days	—	—
Total	245	100 (184)

Note: Most farmers provided more than one use, so each use is treated as independent and percentages are calculated accordingly.

During the survey, 16 cucumber landraces from Kaski and 8 cucumber varieties from Bara were reported with different local names (Box 1). Khatiwada *et al.* (1999) reported 14 varieties in Kaski and 4 in Bara. *Madale*, *Khira*, *Bhakatapure Local* and *Dalle* were common varieties grown in Kaski, whereas *Balma* and *Hariharka* were two common cucumber landraces found in Bara (Box 1). Names of the cucumber varieties are ranked according to their popularity (first being the most common). Seed sellers in Simrounghad market (Bara) sell seeds of *Balma*, *Barshahi khira*, Super green long and Long green cucumber varieties. Similarly AGRO vets based in the same market sell seeds of Green long Super long green, Green long special, Hybrid No. 2, Hybrid No. 3 and Sony #2000 cucumber. The seed of Sony #2000 was brought from Thailand and the rest were from India.

It was not possible to verify the names of the landraces due to lack of references. However, the actual number of varieties mentioned in this paper tallies for Kaski and more for Bara. It seems that all varieties reported in Kaski are landraces, but in Bara at least 3 out of 8 are improved types imported from outside the village. Very few farmers are producing cucumber seed in Bara and most purchased cucumber seed from the market. This indicates that cucumber landraces of Bara may disappear very soon. It was reported that when cucumber was first grown as an off-season crop, its landraces were lost. *Balma* and *Hariharka* used to be very common cucumber varieties in Bara, which are already lost. But farmers still recall these varieties and call other improved varieties by these names based on the fruit characters. In fact, now not a single cucumber landrace exists in Bara, which is a very alarming situation from a diversity point of view and we should strategically plan to overcome this problem at national level.

**Box 1. List of cucumber varieties reported in 2004.****Kaski**

1. *Madale*: thick skin, good for pickle
2. *Khiri*: soft and tasty, fresh use
3. Bhaktapure Local
4. Dalle
5. *Hariyo*: for pickle
6. Majhaura Hariyo Lamo
7. Seto
8. Thulo lamo
9. Kusule
10. *Kalo*
11. Chotto Hariyo
12. Majhaura
13. Chhoto
14. *Lamo*
15. Gada Hariyo
16. Lamo Hariyo

**Bara**

1. *Balma*: more yield, all season type
2. *Hariharka*: used as salad
3. Chichura
4. Dehati
5. Improved
6. Hybrid: high yield
7. *Baishakha*: off-season type
8. *Raini*: improved

**Farmer's opinion**

During the survey, farmers were asked their opinions about cucumber in both sites. Their summarized opinions are given in Box 2. Farmers are looking for better-quality, high-yielding, disease- and insect-resistant cucumber varieties. Farmers are concerned about the seed of the cucumber.

**Box 2. Farmer's expectations/ opinions about cucumber, 2004.****Kaski**

Need for high-yielding, tasty, more fruiting, short and disease-resistant varieties  
Big problem of diseases, no flowering and fruiting, dying plants, deformed fruits (Cucumber mosaic virus)  
Need quality seed  
Need better hormones/medicines, fungicides  
  
Big problem of fruit rot  
Product diversification of cucumber is needed

**Bara**

Difficult to receive seed of *Balma* variety  
  
Need big hilly cucumber  
  
Need quality seed  
Training for seed production and seed storage techniques

**Conclusion**

Cucumber is a versatile and very common summer vegetable in Nepal. Scientific information about Nepalese cucumber is very limited and there is a lack of information on the cucumber seed supply system in Nepal. In Kaski, the traditional seed system for cucumber is still functioning and farmers are maintaining more than 14 cucumber landraces, although some farmers have already started buying cucumber seed from the market. In Bara, the informal seed supply system has already disappeared and a formal seed supply system has been established; however, very few farmers are producing cucumber seed at the village level. This is an alarming situation that needs urgent attention by the concerned bodies to conserve the available cucumber gene pools of Bara site. There is a need to consider the farmers'

expectations on cucumber and tackle their needs and utilization of the landraces for the benefit of the farmers. Loss of the informal cucumber seed system has negative impact, so careful considerations are needed to conserve and use the cucumber diversity for future generations. Raising awareness to strengthen the informal cucumber seed supply system is necessary. The modified approach of FGI is very effective to collect the information in a short period of time, but its accuracy is low because of its methodological procedure and the findings of this study should be used with care.

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## Revealing hidden knowledge on taro (*Colocasia* and *Xanthosoma*) species in Nepal

Deepak Rijal

### Abstract

Nepali farmers have been maintaining rich taro genetic resources and associated knowledge to meet local needs. Although past studies inventoried taro diversity, little has been documented about peoples' knowledge of adaptation, adaptive traits and genotype and environment interactions. We document local knowledge on taro from two mid-hill (670–1400 m) and plain *Terai* sites. Local peoples' knowledge was acquired through household surveys and focus group discussion. Local adaptations are defined as 'relative performance' of genotypes compared with some widely grown cultivars. In taro, local adaptations are assessed for traits that have economic values. Despite location-specific preferences, the common traits are related to tuber yield and eating qualities. Surveys revealed that some taro cultivars are grown away from their original habitats; farmers indicated taro traits that respond when grown away. Like cultivar diversity, farmers have developed location- and cultivar-specific practices. The perceptions regarding effects due to nutrient sources differed between Kaski and Bara farmers. In Kaski, farmers state that organic sources are necessary to produce a tastier corm or cormel. Conversely, Bara farmers never had this experience where taros are fertilized with inorganic sources. Since taro demands high inputs and is grown in large scales, supplying nutrients through organic sources would be impractical, farmers viewed. Clearly, farmers have knowledge regarding the effects of planting practices, availability of soil moisture and soil texture on the performance of economically important traits. Kaski farmers showed particular interest in acidity, corm shape, taste, plant height and cormel size. Unlike in Kaski, Bara farmers always prefer high external inputs for desirable products. This ecological knowledge is shared among farmers during the exchange of planting materials. Empirical studies are recommended to determine adaptability and acceptance of these locally grown taro genetic resources prior to transfer away from their original habitats. To conclude, farmers hold rich ecological knowledge, the foundation of sustainable management of taro genetic resources on-farm.

**Key words:** Taro, descriptor, ecological knowledge, adaptation, adaptive traits, cultivar

### Introduction

Unlike many food crop species, the place of origin for taros is still a matter of further discovery. The earlier scholars have, however, speculated different places of origin for taro (*Colocasia* spp.). The stated regions include South Asia (Lebot and Aradhya 1977), Indo-Malayan, North Eastern India (Plicknett *et al.* 1970; Leon 1977; Cable 1984), Indo-china or West Bengal of India (Leon 1977), North East India and Mainland South East Asia (Purseglove 1972). There are contrasting views on origin and dissemination of rice and taro. Mathews (1995) summarized that rice crop replaced the oldest species like taros (e.g. Harris 1972). Mathews (1998) suggests that taro was the most widely spread starchy species before many modern crop varieties were moved worldwide. Gorman (1977) disagreed with Mathews and proposed that rice and taros were domesticated at the same time from their wild forms adapted to swamps of Southeast Asia. Hence, South Asian farmers hold enormous taro genetic and knowledge resources.

In Nepal, taros (*Colocasia esculenta* L. Schott) have been grown for ages under varied moisture (upland, lowland) and temperature (Mountain, Hill, plain *Terai*) regimes. The earlier researchers have shown a higher number of cultivars found in mid-hills followed by plain *Terai* and high hills (Rijal *et al.* 2001b). The scholars documented direct and positive links between diversities of food traditions, ecological variations and taro cultivars. This diversity was strongly linked to ecological variation, local uses and income-generation for the small-scale farmers.

Farmers characterize taros using a large number of local descriptors related to leaf, petiole, root and tubers. The hill farmers hold enormous ethnobotanical and ethno-ecological knowledge (Rijal *et al.* 2001a, 2001b; Pandey *et al.* 2000). The morphologically variable genetic stocks showed narrow genetic variation on taros (Lebot *et al.* 2003). Unlike this, genetically diverse taro landraces were reported from Nepal (Bajracharya *et al.* 2000; Ochiai *et al.* 2001). Farmers consistently describe taro using local descriptors, which correspond to morphological patterns and isozyme banding patterns (Xixiang *et al.* 2000). South Asian taros are largely triploid (Coates *et al.* 1988), which are reported to have wider ecological adaptation than the diploid species. With Nepal being within the region of taro diversity, Nepali farmers hold rich genetic and knowledge resources. Unlike most self-pollinated food crop species which are largely shaped by farmers (Harlan 1971; Jarvis and Hodgkin 1999), the ways in which farmers manage root crops that are reproduced by vegetative means are poorly documented. In the past, peoples' knowledge related to adaptation, adaptive traits and the effects of ecosystem factors on product quality have not been studied. This paper attempts to reveal peoples' knowledge on taros from Kaski and Bara sites in Nepal. The specific questions addressed in this paper include: (1) How do farmers perceive adaptation on taro? (2) How do farmers describe taro ecosystems? (3) Do farmers describe traits on taro linked to specific environment and/or management factors? (4) How do farmers describe cultivars with regard to adaptation?, and (5) Do farmers' descriptions of soils and crops correspond to those of researchers? The results also have been compared with previous studies.

### **Study sites**

This study was conducted in two contrasting production environments: Kaski and Bara representing mid and low hills. The climate of Kaski is warm temperate to subtropical types where rainfall above 3900 mm is recorded. Bara site, with its subtropical climate, receives lower rainfall but has good irrigation facilities. Temperature decreases with an increase in altitude and vice versa. However, there was no definite distribution pattern for the rainfall. These sites differ in terms of access to road, local crop diversity, markets and infrastructure development. Compared with Kaski, Bara site is more accessible. Further details are found in Paudel *et al.* (2000) and Joshi *et al.* (2003).

These sites differ in terms of ethnic composition, level of food sufficiency, size of land holding, and land fragmentation. In terms of effect of change due to intervention they are different. Unlike Bara, the effect of change on diversity was minimum at Kaski (Upadhyay and Subedi 2000).

The dominant ethnic groups across sites include *Chhetris*, *Brahmin*, and *Baisyas* (Rana *et al.* 2000). Greater land fragmentation was recorded in Kaski (7 parcels per household) than in Bara (4 parcels per household). The average cultivable land holding per household is 0.65 ha and 0.74 ha in Kaski and Bara, respectively. In both the sites, peoples' food and livelihoods are strongly supported by local crop diversity combined with local knowledge.

### **Research methods**

Different techniques were employed to acquire farmers' knowledge. The approaches and methods were chosen according to the type of information to be gathered. For qualitative and descriptive data, participatory techniques were used. The quantitative data were gathered through household surveys. The procedure employed for each technique is presented below.

**Focus Group Discussion (FGD)**

To get collective responses or differences on matters relating to peoples' perception, experience and opinions, FGDs are employed (Nichols 2000). To make discussion effective, participation of 10 farmers as discussants of similar categories was effected. Based on the experience and consultations with local leaders and nodal farmers, diversity-minded older male and female farmers participated in the discussion. These farmers were chosen for their knowledge to participate in wealth-ranking exercise, social and natural resources mapping (Rijal 1998) and farmers' network analysis (Subedi *et al.* 2003). Since this study was part of an ongoing project where researcher were already involved, this work benefited in terms of: (1) identifying farmers holding more ecological knowledge, (2) assessing the distribution of ecosystem, niche and crop diversity, and (3) identifying farmers' descriptors related to soils, ecosystems and crop varieties.

**Household surveys**

Since taros across sites are grown by a limited number of households, a total of 35 randomly selected farmers were interviewed. The questionnaire was developed to document traditional ecological knowledge on taro adaptation and discussed with the enumerators. Prior to field testing, enumerators carried out a pre-test and refined the questionnaire accordingly. The study aims to understand farmer's knowledge on description of crop ecosystems, adaptive traits and local adaptation. Questions on the effects of ecosystem factors on crop performance and product qualities were included.

In both sites, farmers' responses on the effects of local practices, soil moisture, soil texture and chemical fertilizers were documented. The association between local practices and varying economic traits was studied. Farmers' response against each trait was scored as good, medium and poor. The weighted mean for different variables was statistically analyzed. The survey data have been analyzed using descriptive statistics, analysis of variance and weighted mean.

**Results****Crop ecosystems and practices**

The crop ecosystems in which taros are grown vary from location to location (Table 1). Farmers grow taro under three crop ecosystems: sole crop on open fields (*swara bari*), mixed crop and home garden. These ecosystems differ in access to sunlight, soil fertility and soil moisture availability. Compared with open fields, darker (rich in humus and organic matter) and more fertile soils are found in home gardens.

In Bara, taros are mainly grown as a lowland crop under rice fields. Different cultivars are also grown in home gardens at the scale of a few plants. Different crop ecosystems are preferred according to the purpose of production. Home gardens are common across sites where taros are grown for household consumption as vegetable. In Kaski, taros for small-scale production are mixed with other species, e.g. maize, ginger. Conversely, taros grown for market are grown as a sole crop in Kaski (upland) and Bara (lowland).

These systems differ with regard to practices and cultivar type. The practices except for home gardens vary by study sites. Because of the difference in planting methods, associated local practices also differ by study sites. In Bara, taros are grown as a sole crop under deep furrow method with chemical fertilizers and irrigation. Conversely, Kaski farmers grow taros organically following flat methods of planting where mulching and weeding are essentially done. Local perception on these practices was variable (Table 1). Kaski farmers state that local practices are essential if high-quality taros have to be produced. In contrast, Bara farmers state that specific practices are adopted to produce preferred shape and size corms that are preferable to the consumers. To produce the long and attractive corms preferred by local consumers, deep furrow methods are chosen. Taros grown using this

method are said to be desirable. Some cultivar-specific practices were reported in Kaski. Taro cultivars that grow vigorous as well as produce corms such as *Hattipow* and *Khari*, for example, are grown in fertile soils. On the other hand, cultivars that produce cormels and attain shorter heights are cultivated under poor and marginal soils. Such cultivars include *Chaure*, *Khujure* and others.

**Table 1. Household response on local cultivation practices on taro (HH=35 each at Kaski and Bara).**

Local practices	Farmers' response by cropping systems				
	Kaski			Bara	
	Sole	Mixed	HG <sup>†</sup>	Sole	HG
Deep furrow	1	–	4	34	2
Flat planting	24	16	20		2
External inputs (kg/ha)	0.50	–	–	14	–
Irrigation (3–12 times)	1	0	0	34	1
Mulching	20	15		–	1
Organic fertilizer (t/ha)	0.47	0.47	–	–	–
Weeding	5	2	2	–	–

Source: Household surveys, 2002.

<sup>†</sup> HG = home garden.

### Distribution of taro diversity

Taro diversity varied by study sites and crop ecosystems. In Kaski, farmers inventoried 19 cultivars maintained under varied cropping systems (Table 2). The cultivars are grown under sole (n=10,  $H'=0.8532$ ), mixed (n=4,  $H'=0.5775$ ) and home garden (n=11,  $H'=0.9019$ ). The distribution varied in terms of area and local adaptation. Different cultivars under *Colocasia* spp. are primarily grown in open fields. Shanon Index revealed greater value for home garden followed by open fields and mixed cropping. This means that a few cultivars dominate the total diversity maintained under mixed-crop systems. The opposite was true for home garden and monocrop conditions where a large number of cultivars was grown. Compared with mixed cropping, cultivars in sole cropping are grown on larger scales. In Bara, two distinct taro cultivars are grown (*Ujarka* and *Lalka*) which also are grown in home gardens. Recently introduced cultivars were reported as being grown (Table 2). The scale at which taro is grown by species was found to be variable. Cultivars popularly known for petioles and leaves, i.e. *Xanthosoma* spp., are grown on a small scale. Unlike *Colocasia* spp., cultivars belonging to *Xanthosoma* spp. are grown on a small scale (Table 2).

Farmers' opinions were that cultivars can be niche-specific or grown widely across crop ecosystems. In some cases, taro cultivars known for corm and cormel are grown across crop ecosystems. Such widely adapted cultivars include *Panchamukhe* and *Khujure*. Cultivars *Lahure*, *Kalo karkalo* and *Dudhe karkalo* are shade loving and therefore grown in home gardens. Cultivars grown as sole or mixed crop are rarely grown in home gardens. "Here lies the importance of local adaptations to us", farmers stated.



**Table 2. Distribution of taro cultivars cultivated under varying cropping systems (HH=35 each at Kaski and Bara).**

Cultivars	Farmers' response					Area coverage (Ropani) <sup>†</sup>			
	Kaski			Bara		Kaski		Bara	
	Sole	Mixed	HG <sup>‡</sup>	Sole	HG	Sole	Mixed	Sole	HG
<i>Achhame kalo</i>	1	2	0	0	0	0	0.36 (6) <sup>§</sup>	0	0
<i>Dudhe kalo</i>	0	0	3	0	0	0	0	0	0
<i>Dudhe karkalo</i>	0	0	10	0	0	0	0	0	0
<i>Gyante</i>	0	0	0	0	0	0	0.06 (1)	0	0
<i>Hattipow</i>	13	4	2	0	3	0.48 (10)	0.17 (4)	0	0.09
<i>Kalo karkalo</i>	3	0	9	0	0	0	0	0	0
<i>Khari</i>	6	4	0	0	1	0.16 (7)	0.1 (7)	0	0
<i>Khujure</i>	5	0	1	0	0	0.14 (3)	0.15 (2)	0	0
<i>Khujure kalo</i>	1	0	0	0	0	0	0.05 (2)	0	0
<i>Khujure seto</i>	1	0	0	0	0	0	0	0	0
<i>Lahure dudhe</i>	0	0	1	0	0	3 (1)	0	0	0
<i>Lahure kalo</i>	0	0	5	0	0	0	0	0	0
<i>Lahure pindalu</i>	2	0	12	0	0	0	0	0	0
<i>Lahure seto</i>	0	0	7	0	0	0	0	0	0
<i>Panchamukhe</i>	2	0	1	0	0	0.45 (4)	0	0	0
<i>Rato khujure</i>	0	0	0	0	0	0.53 (2)	0.53 (2)	0	0
<i>Rato mukhe</i>	5	2	0	0	0	0.25 (1)	0.09 (5)	0	0
<i>Thaune</i>	0	0	2	0	0	0.09 (2)	0	0	0
<i>Ujarka</i>	0	0	0	31	3	0	0	1.69	0.09
<i>Lalka</i>	0	0	0	2	1	0	0	0	0
<i>Seto pidalu</i>	0	0	0	0	2	0	0	0	0.09
Shanon & Weaver index (H')	0.893	0.577	0.901	—	—	—	—	—	—

Source: Household survey, 2003 (HH=35).

<sup>†</sup> 1 ha = 20 Ropani.<sup>‡</sup> HG=Home garden.<sup>§</sup> Number in parenthesis indicates HH growing particular cultivar.

### Local adaptation and adaptive traits

In general, farmers measure 'adaptation' against economically important traits. Performance of newly introduced varieties is assessed relative to the locally adapted varieties. A few farmers wished to describe adaptation in terms of 'plant health'. A cultivar is considered adapted when its growth in alien environment becomes normal. Adaptations in taros are evaluated against 20 different descriptors, although the number of descriptors used by farmers varied over study sites. In Kaski, 17 descriptors were used and 11 in Bara. Of the total, descriptors were categorized as common (n=8), Kaski specific (n=8) and Bara specific (n=3).

Bara farmers describe and measure adaptation against at least nine descriptors. These descriptors include tuber yield, acidity, infestation of diseases and pests and the number of shoots produced. Cultivars are considered adapted if their performance remains the same or improves under changed environments. In addition, root growth and colour pigmentation are also observed. The Shannon Index (H') revealed that Kaski farmers spread cultivar preference against a large number of descriptors. The Bara farmers are, however, concerned with a few specific descriptors. The analysis shows a direct and positive association between

cultivar diversity and the descriptors used by the farmers. As shown in Table 3, traits such as total yield, acidity, plant height and disease or pest incidence, are more preferred than others. Location-specific preferences are also evident.

**Table 3. Farmers' descriptors used in defining taro adaptation in Kaski (HH=35) and Bara (HH=35) sites, 2002.**

Farmers' descriptor	Kaski	Bara	Total	%	Farmers' descriptor	Kaski	Bara	Total	%
Relative yield	25	35	60	15.5	Corm size	12	0	12	3.1
Acridity	19	35	54	14.0	Plant health	10	1	11	2.8
Plant height	18	18	36	9.3	Growth habit	10	0	10	2.6
Pest attack	11	18	29	7.5	Root growth	0	9	9	2.3
Taste	5	21	26	6.7	Petiole size	8	0	8	2.1
Plant senescence	20	0	20	5.2	Eating quality	7	0	7	1.8
Natural plant colour	13	7	20	5.2	Leaf size	7	0	7	1.8
Cormel count	17	0	17	4.4	Petiole size	7	0	7	1.8
More shoots	0	17	17	4.4	Uniform size	0	6	6	1.6
Corm shape	12	5	17	4.4	Total	17	11	20	100
Leaf senescence	13	0	13	3.4	Shannon Index	1.241	0.778	1.256	NA

Source: Household survey, 2003 (HH=35).

The majority of the farmers referred to specific descriptors that have direct use value: total yield, cormel count, 'staying green' character, eating quality (acridity), corm size and pest infestation. A farmer measured adaptation in terms of maturity duration. To be recognized as a variety, he argues it has to be adapted under local conditions. Some traits are more responsive to ecosystem factors than others, including leaf senescence, acidity, plant height, disease and pest infestation.

Farmers' perceptions regarding local adaptation and adaptive traits over sites were similar (Table 3). Since the majority of the taro cultivars are ecosystem-specific, alternate habitats will have manifold effects on economically important traits. The shade-loving taro cultivars, if grown in open fields, for example, will suffer negative effects, particularly in traits related to cormel, plant height and petiole quality. Shade-loving cultivars are negatively affected, particularly in traits like root growth and eating qualities, when grown to sole cropping. The number of descriptors used was positively associated with the amount of diversity that exists. This may mean that the level of knowledge farmers held depends upon the amount of taro diversity they maintain.

### Plant response to temperature stresses

It is clear that taro grows beyond temperature boundaries from plain *Terai* through to high mountain. Unknown, however, is the degree to which variable temperatures influence the performance of taro. Like other species, taro cultivars are moved across altitudinal gradients along with the peoples' migration, while visiting their relatives and friends within and outside villages. In this regard farmers said that temperature stresses (high or low) can have a variety of effects on plant performance. The effects are directly observed on some specific traits including survival, maturity duration, tuber yield, acidity and cooking as well as eating qualities. However, farmers had mixed responses regarding plant height. The majority argued that plant height increases with an increase in temperature. A few farmers said that plant height and temperatures are to some extent indirectly correlated. There was a common understanding that high temperature can cause adverse effects on corm yield and corm size, but not on qualitative traits like plant colour. Some farmers argued that change in colour to a certain degree is obvious

for plants grown under variable environments. Some farmers referred to the radish crop, which produces the same root colour no matter at which altitude it was grown. Plants exposed to bright sunshine on longer days look greener than those grown as intercrop. After all, adaptation in food crops is evaluated in a relative term, farmers stated.

### **Ecosystem factors and economic traits**

To understand farmers' experience and perceptions regarding local adaptation, questions were asked setting some hypothetical conditions. The possible effects on cultivar performance due to change in growing environments have not been documented. Farmers believed that taro cultivars respond differently when grown away from their original habitat. The degree to which a cultivar responds to new environments varies. Farmers listed 24 descriptors through which effects of different ecosystems factors are examined. Such effects can be positive or negative. The effects are observed directly on shape, size and number of corms, cormels, leaves and petioles. Farmers used plant height, leaf greenness, tuber yield and acidity as other descriptors to measure ecosystem effects. Farmers argued that the size of corm and cormel, number of cormels, and leaf size may be negatively affected when cultivars adapted to open field were grown to mixed cropping systems. Petiole height increases together with their thickness. Some farmers said that leaf colour turns into dark green whereas a few farmers viewed that plant pigmentation remains unchanged. Farmers strongly believed that disease incidence increases. The majority of the farmers felt that effects will be positive on corm size and shape, leaf colour, number and size of the petioles, taste and acidity. Farmers had different experience with cultivars adapted to home gardens when grown under sole crop system. Farmers stated that size of the corm, petiole and cormel increases. Moreover, taste, acidity and plant height may be improved together with reduced disease incidence. Farmers said that the eating qualities might improve (Table 4).

Bara farmers examine effects on at least 18 different traits with economic value. These traits include tuber size and yield, taste and acidity and shoot counts. Farmers' views were gathered on possible effects that might result due to alteration of growing environments. Farmers' experience has been that yield, disease incidence, plant height and seed emergence are improved when cultivars adapted to sole cropping are grown to mixed cropping. Some farmers, however, expressed that corm size may be negatively affected. Farmers stated that tuber yield decreases when cultivars are moved from mixed to sole cropping systems. A few farmers said that the eating quality, disease incidence, cormel size and shape can be improved. At the same time, it was argued that tuber yield increases because of improved access to sunlight.

### **Local practices and variety performance**

In Nepal, taro is grown under various temperature and moisture regimes. In hills and mountains, taros are grown uplands either sole or as an intercrop. Taros in plain *Terai* areas are grown in lowland rice fields as sole crop. Home gardens are common land uses across all the locations. These ecosystems differ in terms of access to light, use of local practices and the types of cultivar grown. In hills and mountains, taros are planted randomly in dibbles with organic fertilizers and mulching. In southern flat areas, taros are planted in deep furrows with the use of inorganic fertilizers and frequent irrigation. It was learned that there are a variety of practices that directly relate to yield and product qualities. The key practices were asked about, together with their possible effects on farmers' preferred traits. The analysis of variance (ANOVA) revealed that the effects of low moisture, clayey soils and low external inputs was statistically insignificant ( $P=0.01$ ). Farmers' responses on the effects of different local practices in each trait were insignificant. In other words, farmers' perceptions regarding the effects on studied traits were similar.

**Table 4. Farmers' perception on environment effects on different traits on taro (HH=35 each at Kaski and Bara).**

Response	If cultivars introduced from:			
	Kaski		Bara	
	Sole-mixed	HG-sole	Sole-mixed	HG-sole
Attractive corm	1	5	–	–
Attractive/greener	–	8	–	–
Large size corm	–	10	–	–
Large size cormel	–	9	–	2
Large size petiole	–	13	–	–
Fewer cormel	8	2	–	1
More cormel	–	7	–	–
Mottle leaf blade	3	3	–	–
Small corm size	20	7	2	–
Small cormel size	11	6	–	–
Small leaf size	10	4	–	–
Decreased acidity	–	3	–	1
Greener leaves	1	3	–	–
High disease	6	1	7	6
Low disease	–	–	6	7
Improved taste	1	3	–	–
Low yield	3	4	7	13
Improved yield	–	–	12	5
Many shoots	–	3	–	–
Medium height	–	11	–	–
Short height	4	–	–	2
Small petiole size	10	12	–	–
Tall height	5	3	4	–
Good taste	–	–	6	8

Source: Household survey, 2003 (HH=35).

The method of planting shows direct impact on corm, cormel, plant height, shoot counts and taste. Farmers responded that greater shoot counts, plant height and biomass yield may be recorded from deep furrow over random planting. Unlike random methods, corm size decreases when grown with deep furrows. The use of mulch causes positive effects on biomass yield, shoot count and plant height as well as corm and cormel qualities. There may be negative effects, however, on the level of acidity and eating qualities. The level of acidity increases for taros grown under excess soil moisture. Farmers stated that optimum moisture condition is required to produce taro with good eating quality, preferable corm size, and desirable plant height and shoot counts (Table 5). The corm size increases with improved soil moisture which may result in a decrease in cormel size. In many aspects, taro grows better in clayey soils that hold more moisture and nutrients. Although taros are cultivated in a variety of soil types, the analysis shows that light soils are not preferred over heavier soils.

Farmers stated that the use of low external inputs will have a variety of effects, particularly on plant height, biomass yield and shoot count. In totality, these were the traits likely to respond to changing environments. Out of several, the main reasons for such changes include: (1) cultivar if grown from open field to home garden or agroforestry, (2) if fertility level changed, (3) if grown in clayey soils, and (4) if local practices change, e.g. mulch, inputs.

**Table 5. Weighted mean estimated against farmers' response on associations between ecosystem factors and key traits, Kaski (HH=35) and Bara (HH=35), 2002.**

Descriptor	Local practices								
	Planting method			Moisture level		Soil texture		External inputs	
	Flat	Furrow	Mulch	Excess	Stress	Clay	Sandy	Low	High
<b>Kaski</b>									
Acridity	4.6	2.0	1.0	2.5	7.2	11.3	3.7	3.0	NA
Corm shape	3.3	9.5	18.0	10.4	12.5	10.4	8.0	6.0	NA
Cormel size	3.5	8.0	17.0	9.0	11.8	10.2	7.5	5.0	NA
Taste	4.7	3.0	2.0	4.8	13.1	9.5	7.0	7.0	NA
Plant height	3.2	8.6	15.0	0.0	8.3	15.3	8.8	5.0	NA
Shoot count	3.0	7.8	20.0	15.5	14.5	12.6	5.4	3.0	NA
Biomass yield	2.9	5.2	18.4	13.9	12.5	12.9	6.8	4.6	NA
<b>Bara</b>									
Acridity	0.0	0.0	0.0	0.0	19.0	19.0	20.0	20.0	20.0
Corm shape	1.7	0.0	0.0	1.6	15.3	17.3	14.4	0.0	13.7
Cormel size	2.0	0.3	0.8	1.2	15.4	16.6	10.3	0.0	14.4
Taste	0.0	0.0	0.0	0.0	17.2	10.0	22.6	19.0	17.0
Plant height	0.0	0.0	2.5	0.7	7.7	5.4	8.4	0.0	5.6
Shoot count	2.0	2.1	3.0	1.0	9.4	13.4	12.2	0.0	10.3
Biomass yield	1.0	0.0	0.0	2.9	6.1	9.0	17.6	5.1	10.9
<i>P</i> values (site)	0.004	0.001	0.005	0.012	0.552	0.631	0.013	0.682	
<i>P</i> values (trait)	0.931	0.376	0.366	0.292	0.651	0.865	0.767	0.483	
SE±	0.43	0.99	2.24	1.44	1.06	0.99	1.52	1.71	

NB: Weighted mean estimated n response x 5 (good or positive effects) + 3 (medium) + 1 (poor or negative)/total Number of responses, Greater score indicates positive associations. 0 value stands for no response.

Similarly, Bara farmers stated that planting methods, moisture status, soil types and sources of soil nutrients significantly affect acidity, corm, cormel and eating qualities. Taros produced from the deep furrow method are considered superior to those produced with the flat method. The practice of flat planting with mulch negatively impacts corm shape and size, shoot count and biomass yield. Performance of corm shape, cormel size, plant height, shoot counts and biomass yield decreases when grown under low-moisture condition. Farmers' experience has been that soil texture can have an adverse effects on taro quality. Compared with sandy soils, taros produced in clayey soils are preferred as they yield a desirable cormel size, taste, plant height and shoot counts. Corm shape, cormel size, plant height and biomass yield are negatively affected by the low level of external inputs. Unlike Kaski farmers, no negative impacts of inorganic fertilizers were reported in Bara.

### Dissemination of ecological knowledge

In Nepal, several networks and subnetworks (Subedi *et al.* 2003) and seed supply systems (Baniya *et al.* 2001) exist through which seed and information are disseminated among farmers. In the past, studies of the kind of information that is disseminated along with planting materials in taro have been few. This study revealed that 28 sets of information are disseminated during exchange of planting materials. These are key traits, which are essentially asked while disseminating cultivars. As listed in Table 6, farmers consider at least 12 descriptors prior to disseminating cultivars. The majority of them are common to farmers of both sites. A few traits can be important to a particular condition. The priority traits for Kaski farmers include uses of plant parts, areas of adaptation and eating qualities. Bara

farmers were specifically concerned with maturity duration, tuber yield and areas of adaptation. This shows that the area of adaptation and local uses were more important than other criteria (Table 6). In addition, information asked together with seed tubers includes corm and cormel characteristics and disease–pest incidence. Thus, farmers include a variety of information while introducing or giving out planting materials through local networks.

**Table 6. Postharvest information disseminated with planting materials, Kaski and Bara (HH=35 each at Kaski and Bara).**

Type of information	Farmers' response	
	Kaski	Bara
Uses of plant parts	20	2
Area of adaptation	19	21
Acridity	17	4
Cooking quality	12	29
Need for mulch	11	—
Yield level	11	35
Planting method	10	6
Pest incidence	10	6
Planting time	10	—
Maturity days	6	23
Market price	—	3
Corm shape	—	2

Source: Household survey, 2003 (HH=35).

### Discussion and conclusions

The distribution of taro diversity varied across both locations and crop ecosystems. The study revealed that greater diversity was recorded in home gardens followed by sole cropping and mixed cropping, indicating that home gardens are important habitats for on-farm conservation. Compared with Bara, a higher number of taro cultivars was recorded in Kaski. Species and cultivars are chosen according to their fitness for local conditions. Cultivars of *Xanthosoma* species are primarily grown in home gardens, whereas cultivars of *Colocasia* spp. are grown under sole as well as mixed crop conditions. The earlier researchers documented that farmers shaped taro diversity as per local needs as well as ecological fits to local conditions (Rijal *et al.* 2001b).

The amount of taro diversity and ecological knowledge are positively correlated. Farmers maintaining greater diversity hold rich ecological knowledge, an indication that there are several networks and subnetworks by which farmers share their experiences among themselves. Prior to dissemination, farmers always explore information regarding crop varieties of interest to them. This study revealed a large number of descriptors used at community level. In actuality, far fewer descriptors are used at household level. Among others, ecological knowledge is generated by visual observation of seed tubers.

The crop ecologists consider species or varieties adapted when they survive and reproduce under given environments (Allard 1997). Crop breeders examine adaptation with reference to a few selected traits, primarily grain yields (Ceccarrelli 1987) whereas forage ecologists assesses adaptation based on species' survival, seed formation and phenological traits (Joshi *et al.* 2001). Farmers describe adaptation in relative terms using a large number of economically important traits. Their considerations were also linked with the complexities of the farming systems. To spread risks and exploit market potentials, featured as in the imperfect markets observed in hills of Nepal, farmers diversify selection parameters. Taro in Bara is produced primarily for the market; farmers give greater priority to a few traits that are economically valuable (yield, adaptation and eating qualities). This result supports the earlier findings (Rijal *et al.* 2001a, 2001b).

Local farmers do have reasons to use location-specific practices and cultivars. Kaski farmers' experience has been that taros grown organically are tastier than those produced with inorganic fertilizers. The use of inorganic fertilizers provides a simple solution if taro is grown to secure a good yield. It is argued that inorganic fertilizers are used as alternative sources when organic manures are unavailable. Farmers have different reasons for growing taros in dibbles with mulching: it suppresses weed growth and protects the crop from early season drought. Bara farmers, however, showed ignorance about such benefits although they have been growing taro for decades. Instead farmers stressed that taro for the market is grown with inorganic fertilizers and irrigation. The deep-furrow method is chosen to produce the preferred corm (long and uniform size). Seed tubers are placed in a 30–40 cm deep trench and earthing up is done to check luxurious growth. Producers have not received any negative response on their produce from consumers.

There was a common agreement among farmers that quality traits are cultivar-specific; their expressions, however, are influenced by environment and management factors. The experimental results revealed that the plots with mulch and organic fertilizers yielded significantly more than plots with herbicide weed control and inorganic fertilizers (Miyasaka *et al.* 2001). In another study, the use of inorganic fertilizers showed no marked difference in marketable corms; however, non-marketable corm yield was significantly increased. Higher biomass yield was produced with the use of inorganic fertilizers. The efficiency by which the applied N was used was low and fertilizer recovery was only 10% in taro, suggesting that fertilizer N failed to increase yield of taro (Hartemink *et al.* 2000). Percent dry matter was enhanced with organic fertilizers with mulch over inorganic fertilizers. The use of mulch results in a significant increase in total N, organic C, exchangeable K, Ca, Mg with compost and silage mulch than plots with wood and only silage mulch (Miyasaka *et al.* 2001). Despite increased N and Ca levels, foliar concentrations of N and Ca were lower in taro grown in mulched plots compared with non-mulched plots. This could be due to growth-dilution effects (Jarrell and Beverley 1986). This finding supports the earlier conclusions suggesting that farmers' ecological knowledge is relevant.

### **Implications**

Traditionally taro has been supporting the nutrition foundation for people of the Pacific Islands (Kahn 1988; Bayer 2003; Pollock 2003), India (Edison *et al.* 2003), China (Jianchu *et al.* 2001), Japan (Mathews 1995), Vietnam (Hue 1998; Hue *et al.* 2003) and Nepal (Rijal *et al.* 2001b). In recent times, taro is gaining popularity in New Zealand (Bussell *et al.* 2003). Farmers as custodians hold rich ecological knowledge of crops that are vegetatively propagated such as taro. It is clear that the amount of farmers' ecological knowledge and diversity go together. Unlike *Tera*i farmers who maintain a few cultivars, hill farmers hold more ecological knowledge where the higher number of cultivars was grown. Taro diversity also varied by ecosystem.

A higher number of taro cultivars was recorded in home gardens than in mixed or sole cropping. Likewise, location-specific practices and perceptions are created according to their fit to local conditions. The strength of local knowledge and practice is that they are locally adaptable as well as socially acceptable. Introduction of promising cultivars along with the information related to their uses provides farmers with options, thereby enhancing on-farm conservation. Conservation priority may be focused for ecosystems that are rich in taro diversity, e.g. home gardens. The promotion of nutritionally rich species with high yield potential such as taro can help strengthen human food and nutritional securities. Further work is required to establish a scientific foundation behind local knowledge and practices before they are widely disseminated. Since these resources provide valuable foundations for present and future work, their integration into research and development programmes is inevitable.

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## Locating diversity of broad leaf mustard (*Brassica juncea* L.) in Nepal

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### Abstract

Nepal is rich in crop diversity and this diversity includes a large number of local leafy vegetable species. Broad leaf mustard (BLM), locally called *Rayo*, is a common and important vegetable in hilly areas. Owing to its wide range of adaptability and typical taste, it is one of the indispensable leafy items in a daily diet. Literature reviews and key informant surveys show that a large number of collections of BLM have been made in the past with the objective of evaluating the superior landraces with more yield, soft leaf and late bolting. A total of five local landraces of BLM have officially been released as commercial cultivars. Resistance breeding against Turnip Mosaic Virus (TMV) also has been successful in developing a cultivar named 'Manakamana'. A large BLM diversity exists in Nepal, especially across the mid-hill. But the growth of commercial vegetable production pockets and development of new link roads have resulted in genetic erosion of BLM landraces.

**Key words:** Broad leaf mustard, diversity, genetic erosion, Nepal, *Brassica juncea*

### Introduction

Broad leaf mustard (BLM) (*Brassica juncea* L.), which is *Rayo* in Nepali, is one of the most popular and traditional vegetable crops with a high degree of diversity (Pandey 1995; ARS 2000). It is a highly praised winter leafy vegetable, widely grown for its peculiar taste. A large BLM diversity exists in Nepal especially across the mid-hills. Since the inception of vegetable research in the country, collection, evaluation and recommendations of these local landraces of broad leaf mustard have been made and have identified the superior landraces, which were released for general production (Pradhanang *et al.* 1993; Panthee 1998). Evaluation of local germplasm was done in Lumle (Pradhanang *et al.* 1993), Pakhrisbas and Khumaltar before 1990.

At present, five local landraces have been released for commercial cultivation by national variety release committee after a series of field evaluations. These released cultivars are Khumal Broad Leaf, Khumal Red Leaf, Marpha Broad Leaf, Manakamana and Thankhuwa Local. The name of the varieties itself indicates the site of collection of these landraces Khumal, Marpha, Manakamana and Thankhuwa. Another local collection from Eastern Hill, Thankhuwa, Dhankuta has been registered under the Variety Registration Committee and is in the process of being officially released. Although landraces collected from Nepal have been extensively used in breeding programmes in Hong Kong and China and several varieties have been developed, use of locally adapted materials in Nepal is limited.

Because of the commercialization in vegetable production, many commercial production pocket areas have been developed. In these areas, farmers grow and produce the hybrid cultivars. However, it is also true that vegetable cultivation is emerging as one of the important cash-generating activities. The trend is more visible towards the different road corridors of the country. So, a threat exists for the erosion of resources of this valuable vegetable, BLM, and the rapid loss of this diversity is a national concern today. The maintenance and selection of local BLM has been rapidly disappearing because of easy access to seed from seed entrepreneurs (Agrovets) and few improved landraces are available. The lack of knowledge about the important diversity-rich areas of BLM and their socioeconomic dimensions has constrained the informed management of valuable genetic resources for BLM. To conserve these valuable resources, locating diversity-rich areas of broad leaf mustard is one of the prime and most important activities that will support development and implementation of conservation strategy and action.

### **Materials and methods**

Available literature was reviewed with the relevant information and passport information about the collections. On the basis of the review, a checklist was prepared to conduct a key informant survey with the concerned authorities who have been involved in either collection, evaluation or release of the BLM cultivars in Nepal. The key informants were also identified. A total of five individuals were contacted to discuss information included in the checklist. On the basis of information received from the literature survey, key informant survey and personal experiences of farmers, the results were summarized and some suggestions and conclusions were drawn for the conservation and utilization of broad leaf mustard germplasm.

### **Results and discussion**

#### **Varietal development work**

Efforts in the past were made to collect, characterize and evaluate BLM germplasm by various organizations. The Vegetable Development Section, Khumaltar did the pioneer work in collection, evaluation and recommendation with the help of the FAO-funded "Fresh Vegetable and Vegetable Seed Production Project". Collection was made from the eastern, central and western hills of Nepal (Figure 1). The general features of the released cultivars of BLM are given in Table 1. The unique characteristic of Marpha Broad Leaf is that it produces very tender leaves for a long duration with very good taste and it is very slow to bolt in mid-hill (800-1400 m), river basin and plain areas (<800 m) of Nepal. Other cultivars are of mid-hill origin. They are, however, earlier bolting than Marpha Broad Leaf. Both the Khumal cultivars—*Khumal Chauda Pat* (Khumal Broad Leaf) and *Khumal Rato Pat* (Khumal Red Leaf)—were selected from the populations of BLM grown in the vicinity of Khumaltar, Lalitpur (1350 m). Khumal Broad Leaf has large, broad leaves with dark green colour whereas the leaf of *Khumal Rato Pat* Leaf is purplish, has a purple midrib and is rich in vitamin A. The cooking quality and taste of these cultivars are lower than those of Marpha Broad Leaf.

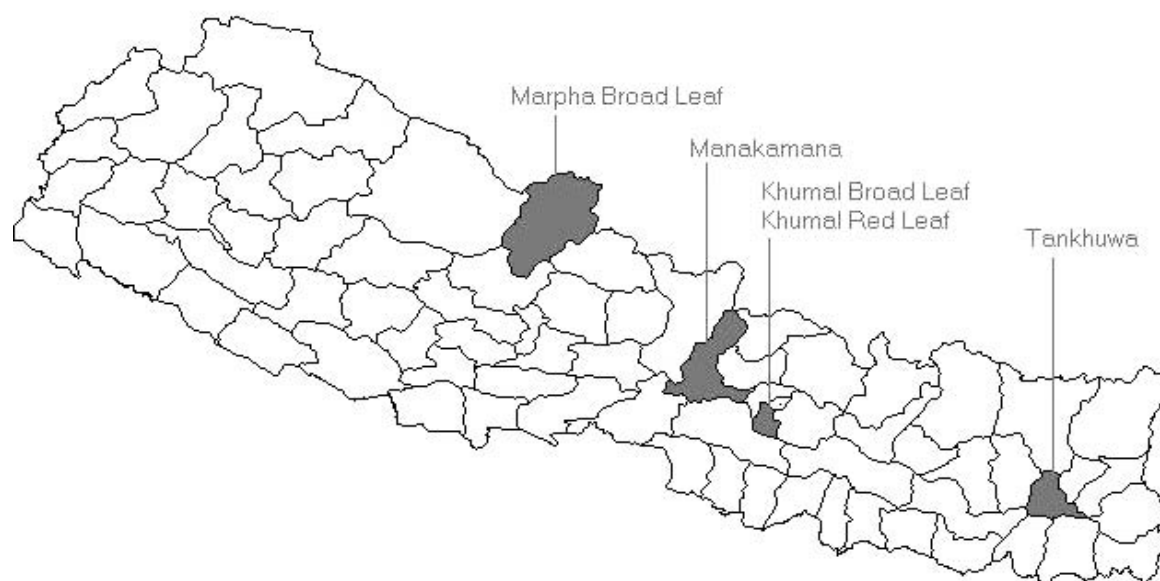
Manakamana is another cultivar selected from the populations of BLM from Manakamana (1150 masl) of Gorkha District, in the western part of Nepal. It has coarse leaf surface and a slight hard leaf texture. Thankuwa Local is a selection made from the BLM population, which was collected from Thankuwa (1300 m) of Dhankuta District, Eastern Nepal. The leaves are light green colour, very tender, smooth in texture and the variety is late bolting (Table 1). Late bolting is the trait most preferred by farmers for *Rayo*.

#### **Collection, characterization and evaluation of BLM landraces**

Panthee (1995) developed and used BLM descriptors for the characterization of BLM germplasms. For the characterization of collected BLM germplasms, the major characters used were seedling morphological traits, anthocynin pigmentation, growth habit, plant height, leaf morphology, flower morphology, seed morphology, etc.

Resistance breeding against Turnip Mosaic Virus (TMV) was conducted at Lumle, Kaski during the late 1990s, crosses were made and progenies were forwarded. Performance studies of  $S_4$  and  $S_5$  progenies of BLM cv. Manakamana were carried out and, finally, the cultivar Manakamana was officially released as a TMV-resistant cultivar (Ghimire and Sharma 1997; Panthee 1998).

The Horticulture Research Division, Khumaltar has a collection of 15 local and exotic germplasms and 5 improved cultivars in their regular evaluation process (HRD 1997). The programme has been discontinued and the seeds are no longer viable. Nepali *et al.* (2000) reported a local landrace of BLM called Mujure from Ghalegaoun (2070 m) of Lamjung district, which is grown during winter season and is a popular vegetable in that area.



**Figure 1. Site of collection of released cultivars of BLM.**

**Table 1. Some characteristic features of the released cultivars of broad leaf mustard.**

Cultivar	Maturity type	Days to maturity	Leaf colour	Plant height (cm)	Leaf size (cm)	Yield (t/ha)
Khumal Broad Leaf	Medium early	40–50	Puckered dark green	45–50	25–30	25–30
Khumal Red Leaf	Late	60–70	Puckered light red	25–30	20–25	25–30
Marpha Broad Leaf	Late	55–65	Puckered light green	40–50	20–30	25–30
Tankhuwa Local	Early	33	Smooth light green	40–45	17–30	20–25
Mankamana	–	–	Whitish green	–	26–46	–

Source: VDD/SEAN 1995.

Efforts also were made by Pakhribas Agriculture Center to collect and evaluate BLM landraces from Koshi hills. Although the number and details of entries were not reported, two local landraces from Muga and Thankhuwa were emerging as candidate varieties with comparatively good yield for recommendation.

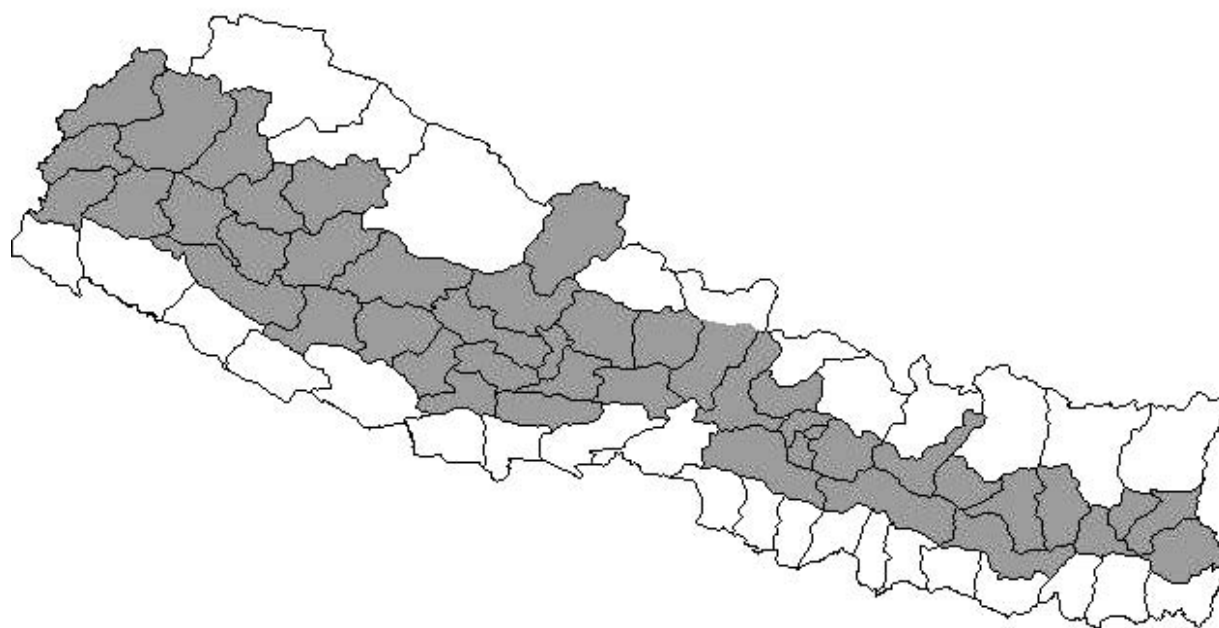
### **Distribution of broad leaf mustard germplasm**

Primarily BLM is a cold-season winter crop and requires low temperature (chilling) exposure for its bolting. Depending upon the place of origin, duration for exposure to low temperature for bolting varies among the varieties. In Manakamana, bolting starts when temperatures start rising whereas it is very hard to bolt in Marpha Broad Leaf under lower altitude environment.

This indicates that BLM is distributed throughout the country, especially in mid-hill (1000–1400 m) to Trans-Himalayan zones (>2200 m) like Marpha, Mustang. From the Trans-Himalayan zone, only one local landrace has been officially released for wider cultivation and this is still one of the most popular cultivars. Agriculture Botany Division has 11 different farmer-named landraces (Upadhyay and Joshi 2003).

From east to west, a large number of BLM landraces have been found (Figure 2) and much indigenous knowledge exists (Budathoki *et al.* 1993; Pandey 1993). In commercial production pockets, all these five cultivars are popular depending on the location. In kitchen gardens, farmers maintain their own seeds of BLM for next season plantings. For home consumption, farmers maintain a few plants of BLM in the back of a cattle shed or in the heap of farmyard manure to produce seed and store for the next season.

The plant characteristics and morphology have been found to be different in different locations. In eastern mid-hill, Thankhuwa type BLM is widely cultivated, whereas in central mid-hill, Khumal Broad Leaf types are dominant. In midwestern hills, both Khumal Broad Leaf type and Khumal Rato (Red) types are grown by farmers.



**Figure 2. Possible areas (shaded) where diversity of broad leaf mustard exists.**

#### **Organizations involved in BLM germplasm collection and utilization**

The following organizations have been involved actively in collection, characterization, evaluation and conservation of BLM germplasm:

- Vegetable Development Division, Khumaltar, Lalitpur (Released 3 cultivars)
- Lumle Agriculture Center, Lumle, Kaski (Released 1 cultivar)
- Pakhribas Agricultural Center, Pakhribas (Collected 45 landraces and released 1 cultivar)
- Agriculture Botany Division, Khumaltar, Lalitpur (Collected and maintained 136 accessions from different agroecological zones and have maintained the passport data)
- Horticulture Research Division, Khumaltar (Collected 15 landraces including two modern varieties).

#### **Reasons for eroding genetic diversity of BLM**

Owing to the introduction of different released cultivars and modern varieties, farmers have been choosing to cultivate these varieties despite the existence of their own landraces, especially in commercial vegetable production areas. However, in non-commercial areas farmers are still maintaining their own landraces.

The increase in area under commercial vegetable production, especially in mid-hills, has resulted in threats to many local landraces in the region. The areas that are accessible to roads are now being converted into commercial vegetable production pockets. The construction of new highways (e.g. Karnali Highway), different link roads to the main highway and agricultural roads inside the district have turned the traditional farmlands into commercial vegetable pockets (Figure 3).



**Figure 3. Road network of Nepal where commercial vegetable production pockets exist.**

### Conclusions

Mid-hills (800–1400 m) are the major areas where a large amount of BLM genetic diversity exists and many landraces have been collected. No systematic methods in collection, characterization and conservation have been adopted in the past by the organizations involved in those activities. However, some work has been done on a parcel basis by organizations other than the authorized organizations.

There is a continued threat to genetic erosion of BLM landraces. Increase in commercial vegetable pockets along the road corridors, introduction of hybrid cultivars, and abandonment of practices of seed production by farmers for their own crops are the basic causes for genetic erosion. Commercial farming is a kind of monoculture as compared with traditional farming, where there is a lot of crop diversity. The reduction in crop diversity certainly threatens the existence of local landraces. The areas being commercialized in mid-hill are the most potential area for BLM diversity, where attention should be paid to the conservation of BLM germplasm.

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## Characterization and genetic diversity of mango (*Mangifera indica* L.) in Nepal

Abishkar Subedi, Jwala Bajracharya, Bal K. Joshi, Hom N. Regmi, Salik R. Gupta and Hari B. KC

### Abstract

Mango (*Mangifera indica* L.) is an important tropical fruit of Nepal and occurs as a domesticated or wild entity in the complex biotic community of the ecosystem. Wide ecological variation and diverse needs of multi-ethnic communities have enriched the country with genetic wealth of diverse mango varieties as landraces and this diversity is considered a valuable economic asset for on-farm conservation in Nepal. Siraha, Saptari, Dhading, Kavre, Parbat, Baglung and Dadeldhura districts representing the tropical and subtropical ecosystems were surveyed to collect and characterize the diversity of mango. A total of 216 fruit samples and 23 young leaf samples of mango landraces were collected and evaluated for 19 qualitative and quantitative morphological and isozyme characteristics to compare the genetic variations and relationships. A range of variation existed in mango for fruit with different shapes, colour, sizes, flavour (aroma) and taste with 25–47 coefficients of variation and 0.592–0.865 Shannon Weaver index. Siraha and Dadeldhura are rich in mango diversity. Principal component analysis of qualitative traits and quantitative traits of fruits showed the existence of a continuum of mango diversity in Nepal with no ecogeographic differentiation. Qualitative traits were of importance in determining the groupings and relationships. Esterase isoenzyme profiles and cluster analysis based on allele frequencies revealed genetic variation (0.33–1.00 Jaccard's similarity coefficient) and showed relationships among farmers' varieties. The observed variation in morphological and esterase isoenzyme of mango is discussed in the context of on-farm conservation.

**Key words:** Mango, genetic diversity, landraces, diversity index, esterase, isozyme banding pattern

### Introduction

Mango (*Mangifera indica* L.) is an important tropical fruit of Nepal and occurs as a domesticated or wild entity in the complex biotic community of the ecosystem. Wide ecological variation and diverse needs of multi-ethnic communities have enriched the country with a genetic wealth of diverse mango varieties. The history of mango cultivation can be traced back thousands of years (Pandey 1998). Since mango, in particular, with its nutritious and delicious fruit has been such an important fruit tree, the landraces grown on-farm are important resources for utilization in future. The global project on "Strengthening the scientific basis of *in situ* conservation of agricultural biodiversity on-farm, Nepal component" identified mango as a mandatory crop for an understanding of the extent of its diversity and its on-farm conservation.

The home gardens of tropical plain, river gorge areas and subtropical valleys are the common habitats of Nepalese mango diversity. However, in recent years the original habitats of mango have changed in response to biotic, economic and other pressures. For example, many historical mango orchards have been threatened by logging and modern agriculture system. Despite the significant contribution to livelihoods of the people, no proper assessments of diversity and associate knowledge have been documented. There is a lack of precise information about the current status of local germplasm or landraces, location of diversity-rich areas and the ways farmers manage and utilize the crops. Quantitative and qualitative morphological characterization, biochemical electrophoresis and molecular methods have been widely applied in measuring the level of variation within and among



populations and have provided useful information on the structure of the genepool in a large number of plant species (IBPGR 1989; Hamrick and Godt 1990; Kashkush *et al.* 2001). Qualitative traits of fruit in Bush mango (*Irvingia gabonensis*) were important traits for assessment of levels of genetic variation (Leakey *et al.* 2000). Decha (2004) found morphological characterization and isozyme as useful traits in identifying the mango clones from eight provinces of Upper North India. Fruit characterization and isozyme analysis were used in the present study to characterize the diversity of mango landraces grown in farmers' fields in potentially diversity-rich areas in Siraha, Saptari, Dhading, Kavre, Parbat, Baglung, and Dadeldhura representing the tropical and subtropical ecosystems of the country.

## Materials and methods

### Materials collected for morphologic traits

Following the review of relevant literature and consultation with the mango experts, Siraha, Dhading, Kavre, Parbat, Baglung and Dadeldhura were the districts selected as sites for ecogeographic survey and were assumed rich in mango diversity (Dr K Budhathoki, pers. comm.; Subedi *et al.*, p. 176, this volume). The districts represented the different agroecosystems ranging from 100 to 1450 m (Terai to mid-hill) and 216 accessions of mango varieties with different traditional names as farmers' unit of diversity (FUDs) were collected and characterized for genetic diversity analysis based on morphological characters of fruit (Table 1). Four to five fruits per accession were collected from each collecting site during an ecogeographic survey of mango in 2003.

**Table 1. Number of accessions, fruits and leaf samples of mango varieties collected from different districts for morphological characterization.**

Region	Districts	Altitude (m)	Accessions (n)	Fruit samples	Farmers' unit of diversity (FUDs)
Eastern	Siraha	100–150	203	186	62
Central	Kavre	850–900	143	102	31
	Dhading	600–1050	134	109	34
Western	Parbat	700–950	123	92	29
	Baglung	750–950	74	36	10
Far western	Dadeldhura	300–1450	150	72	21

### Materials for isozyme variation

Young leaf samples of 23 accessions of mango landraces from nursery-raised trees at the Regional Agriculture Research Station, Tarahara, were evaluated for isozyme analysis. These accessions were collected from Siraha (Lahan, Mirchaiya) and Saptari (Sripur) Eastern districts under a local mango germplasm collection programme of Tropical Fruit Tree (TFT) project by Horticulture Research Division, Khumaltar (Table 2).

### Fruit characterization

A checklist consisting of both qualitative and quantitative characters was prepared through review of IBPGR descriptors for mango (IBPGR 1989), thematic group discussions and personal communication with Dr Budhathoki (Table 3). Collected fruit samples were evaluated for variation in fruit qualitative and quantitative traits in the research laboratory of LI-BIRD. Data were entered in a checklist and computerized for further analysis.

**Table 2. Landraces (FUDs) used for isozyme analysis.**

Landrace	Accession	Number of samples	Collection site
<i>Beluwa</i>	KS9	3	Sripur, Saptari
<i>Bathiya</i>	KS2	3	Sripur, Saptari
<i>Sinduriya</i>	SMR17	3	Mirchaiya, Siraha
<i>Jhokiya</i>	KS27	4	Sripur, Saptari
<i>Pohoriya</i>	KS8	4	Sripur, Saptari
<i>Laduwa</i>	KS16, SMR2	4,3 (7)	Sripur, Saptari; Mirchaiya, Siraha
<i>Supariya</i>	SMR18	3	Mirchaiya, Siraha
<i>Khairawa</i>	KS13	3	Sripur, Saptari
<i>Bhadaiya</i>	SLM5, SMR12	3,4,3 (10)	Lahan, Mirchaiya, Siraha
<i>Sarahi</i>	SLM8	3	Lahan, Siraha
<i>Misrikant</i>	SLM6	4	Lahan, Siraha
<i>Darmi</i>	SLM15	5	Lahan, Siraha
<i>Biju</i>	SMR10	2	Mirchaiya, Siraha
<i>Bijusarahi</i>	SLM11	2	Lahan, Siraha
<i>Krishnabhog</i>	KS22	4	Sripur, Saptari
<i>Motihari</i>	SLM3	5	Lahan, Siraha
47		3	Not known
57		3	Not known
58		4	Not known
59		4	Not known
Total		79	

**Table 3. Quantitative and qualitative characters of fruits recorded in the study.**

Quantitative characters	Qualitative characters
Fruit weight (g)	Fruit shape (3 states)
Fruit length (cm)	Skin colour (3 states)
Fruit width (cm)	Flesh colour (4 states)
Fruit thickness (cm)	Fibre content (3 states)
Skin thickness (cm)	Fruit aroma (2 states)
Flesh thickness (cm)	Pulp taste (3 states)
Seed length (cm)	Pulp colour (4 states)
Seed width (cm)	Seed shape (4 states)
Seed thickness (cm)	
Seed weight (g)	
Brix (%)	

### Isozyme analysis

Equal proportions of 4–5 young leaves of the clones of mango accessions raised in a nursery in Tarahara field condition were extracted in L-Ascorbic acid buffer (0.1 mol/L ascorbic acid, glycerol, and pH 7.4) following the methods of GEVES (GEVES 1993). The samples were electrophoresed in tris buffer (0.400 mol/L Tris, pH 8.0 with 105 mol/L citric acid) using a 12% starch gel (potato starch S-4501), Sigma company, USA). Four enzymes were assayed by activity staining dipping the gel slices in respective enzyme trays, and enzymes for staining were prepared following Cheliak and Pitel (1984). Three enzymes (MDH, ADH and PRX) could not be scored and isozyme results drawn in the present study are based only on the esterase (EST, E.C.1.1.1.2) scorings. Genetic interpretation of the esterase banding pattern could not be done but we scored each individual band seen as presence (1) and absence (0) for further analysis of the genetic similarity (Wendel and Weeden 1989).

### Data analysis

Three fruits of each accession were characterized for 11 quantitative and 8 qualitative traits following IBPGR descriptors for mango. Descriptive statistics of quantitative traits, two

diversity indices (Shannon-Weaver and Simpson) of qualitative traits across the collection sites (Shannon 1948; Magnussen and Boyle 1995) were estimated. Analysis of variance and principal component analysis (PCA) using both quantitative and qualitative traits were performed. Significantly different quantitative and qualitative data were standardized for PCA. The variability accounted for by each of first two axes of ordination was plotted for each site of collection and together for all sites. The importance of traits in determining the genetic variation among the accessions under study was ascertained with their loading values.

A binary data matrix was created for numerical analysis of isozyme traits. Three bands were detected and each band used as individual isozyme character (electromorph) for measuring the variation within and among mango landraces under study. Zymograms were constructed based on the combination of these bands. A pairwise similarity matrix was calculated using Jaccard's similarity coefficient and a dendrogram was constructed by the use of Unweighted Pair Group Method with Arithmetical Averages (UPGMA) following NTSYSpc (Rohlf 1990).

## **Results**

### **Quantitative traits**

A remarkable variation among the mango accessions across the surveyed districts was revealed in the means of fruit characters considered (Table 4). The analysis of variance determined significant differences among the accessions of mango collected from different districts for 11 quantitative traits of fruit (Table 4). Coefficient of variation was high for most quantitative characters of fruit among the accessions within each collection district. A great range of variation was encountered in fruit weight and length among the accessions within and between ecogeographically surveyed districts. Fruit and kernel (seed) measurements were significantly variable among the mango accessions collected in the regions.

### **Qualitative traits**

Great difference in diversity index of mango based on fruit quantitative traits revealed the existence of diversity in mango in the collected regions (Table 5). Fruit shape, flesh colour, pulp taste and seed shape were found significant for assessment of the level of variation in mango landraces. Highest diversity index was measured in accessions from the eastern region (Siraha) and least in accessions from the western region (Baglung). In particular, the qualitative trait skin colour was rich in extent and distribution of diversity across the regions with high values of Shannon and Simpson indices. However, the average diversity and evenness of diversity indices of the six districts representing four regions of the country were Siraha (0.87, 0.51); Dadeldhura (0.79, 0.43); Dhading (0.73, 0.45); Parbat (0.64, 0.40); Kavre (0.62, 0.39) and Baglung (0.59, 0.37).

### **Clustering and relationships**

Principal component analysis of 4 qualitative and 6 quantitative characters data with significant variations revealed the existence of a diversity continuum in mango and no ecogeographical or regional pattern of distribution was observed. The ordination clearly showed Siraha, Dhading, Kavre (Paanchkhal) and Dadeldhura with similar distribution of diversity along two axes in equal distances, whereas those of Parbat and Baglung showed a similar pattern of distribution along the axis 2. This pattern is clearly seen in Figure 1, when same ordination is replotted for the districts separately. The accessions from Eastern (Siraha), central (Dhading) and Far-Western (Dadeldhura) regions clustered together and quantitative data were found influential for it (Table 6). For the accessions from Western region (Parbat and Baglung), the qualitative data were most influential in clustering them together (Table 6).

**Table 4. Means and coefficient of variation (%) of quantitative characters of each mango fruit collected from different regions of the country.**

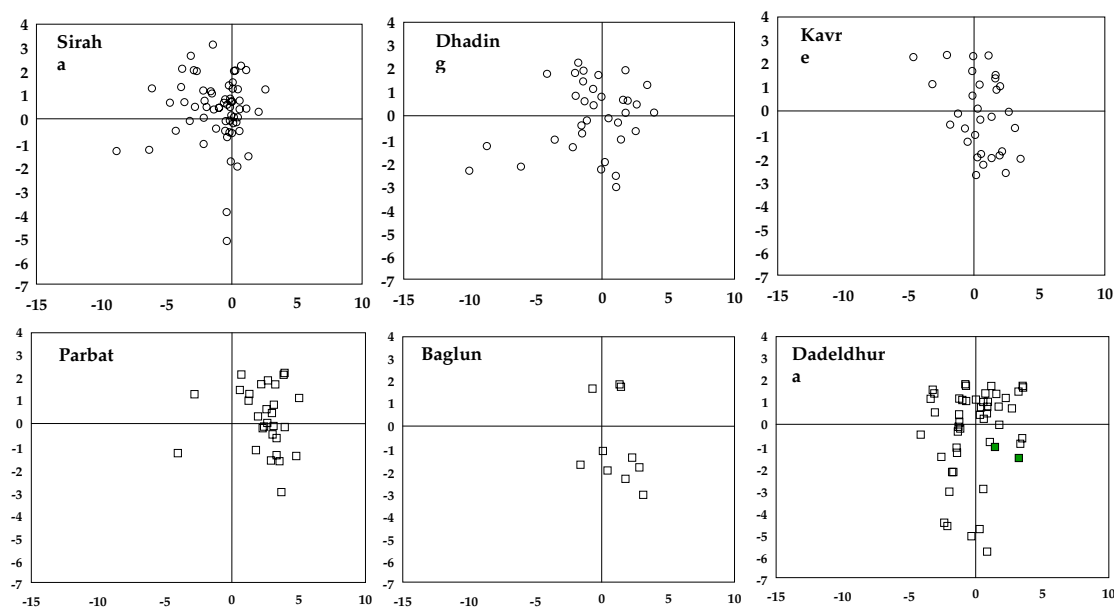
Character		Districts						F value
		Siraha (62)	Dadeldhura (50)	Dhading (34)	Parbat (29)	Baglung (10)	Kavre (31)	
Fruit weight (g)	M†	217.5	160.7	206.0	69.4	101.2	133.9	1.10 ns
	CV‡	0.85	0.61	1.01	1.50	0.60	0.58	
Fruit length (cm)	M	8.9	8.2	8.3	5.8	7.0	10.1	1.55**
	CV	0.23	0.21	0.33	0.29	0.18	1.89	
Fruit width (cm)	M	6.5	5.7	6.2	4.3	5.0	5.6	1.55**
	CV	0.23	0.23	0.28	0.27	0.16	0.18	
Fruit thickness (cm)	M	6.0	5.4	5.8	3.9	4.6	5.2	1.42*
	CV	0.19	0.23	0.26	0.31	0.20	0.19	
Skin thickness (cm)	M	0.21	0.22	0.22	0.17	0.25	0.21	0.78 ns
	CV	0.34	0.42	0.25	0.30	0.21	0.17	
Flesh thickness (cm)	M	1.9	1.6	1.7	1.2	1.3	1.6	2.33***
	CV	0.30	0.32	0.37	0.17	0.21	0.23	
Seed length (cm)	M	7.2	6.8	6.9	4.9	5.9	6.2	1.66 **
	CV	0.20	0.21	0.33	0.30	0.19	0.26	
Seed width (cm)	M	3.5	3.3	3.6	2.8	3.2	3.2	1.10 ns
	CV	0.18	0.18	0.28	0.19	0.14	0.14	
Seed thickness (cm)	M	2.1	1.9	2.0	1.9	2.0	1.9	2.19***
	CV	0.17	0.17	0.17	0.19	0.20	0.25	
Seed weight, g	M	27.0	22.4	28.4	17.1	22.1	18.9	1.27 ns
	CV	0.37	0.34	0.63	0.42	0.39	0.58	
Brix, %	M	16.6	15.2	14.2	14.8	13.4	15.6	0.88 ns
	CV	0.24	0.29	0.24	0.22	0.31	0.23	
<b>Average</b>	<b>CV</b>	<b>0.30</b>	<b>0.29</b>	<b>0.38</b>	<b>0.36</b>	<b>0.23</b>	<b>0.43</b>	

† M = mean. ‡ CV = coefficient of variation. Figure in parenthesis is sample size.

**Table 5. Shannon Weaver and Simpson indices based on qualitative traits showing diversity of fruit types across the collection sites.**

Character	F value	Districts					
		Siraha (62)	Dadeldhura (50)	Dhading (34)	Parbat (29)	Baglung (10)	Kavre (31)
Fruit shape	2.58*	0.73(0.39)†	0.59 (0.31)	0.30 (0.16)	0.33 (0.19)	0.00 (0.00)	0.24 (0.12)
Skin colour	1.77 ns	1.07 (0.65)	1.03 (0.62)	1.01 (0.61)	1.03 (0.63)	1.03 (0.62)	0.73 (0.46)
Flesh colour	4.97***	0.83 (0.46)	0.84 (0.48)	0.81 (0.49)	0.55 (0.37)	0.61 (0.42)	0.68 (0.49)
Fibre content	2.67	0.55 (0.61)	0.37(0.63)	0.61 (0.60)	0.46 (0.53)	0.50 (0.56)	0.53 (0.64)
Fruit aroma	0.03 ns	1.01 (0.37)	1.05 (0.21)	1.00 (0.42)	0.87 (0.29)	0.95 (0.32)	1.05 (0.35)
Pulp taste	0.23 ns	0.88 (0.53)	1.03 (0.62)	0.96 (0.58)	0.80 (0.51)	1.03 (0.62)	0.91 (0.55)
Pulp colour	5.12***	0.86 (0.50)	0.85 (0.50)	0.65 (0.46)	0.68 (0.49)	0.61 (0.42)	0.67 (0.48)
Seed shape	8.23***	0.99 (0.54)	0.60 (0.09)	0.49 (0.26)	0.40 (0.19)	0.00 (0.00)	0.14 (0.06)
Mean		0.87 (0.51)	0.79 (0.43)	0.73 (0.45)	0.64 (0.40)	0.59 (0.37)	0.62 (0.39)

† = Figure in parenthesis is the Simpson index; ns = not significant; \*p = >0.05; \*\*p = >0.01; \*\*\*p = >0.001.



**Figure 1. Scatter plot of mango accessions to show the distribution pattern in various districts.**

**Table 6. The morphological characters with their loadings for Axis 1 and Axis 2 in the ordination.**

Quantitative character	Loadings		Qualitative character	Loadings	
	Axis 1	Axis 2		Axis 1	Axis 2
Fruit length	-0.236	+0.074	Fruit shape	-0.090	+0.011
Fruit width	-0.498	+0.018	Flesh colour	-0.060	-0.697
Fruit thickness	-0.485	+0.020	Pulp colour	-0.034	-0.700
Flesh thickness	-0.390	+0.088	Seed shape	-0.229	-0.073
Seed length	-0.452	-0.004			
Seed thickness	-0.197	+0.064			

### Isozyme traits

A single zone was detected as active in staining with EST across 23 farmers' varieties collected from Siraha and Saptari, two Eastern Terai districts. Three bands were scored and found polymorphic. Based on the combination of these bands, 6 banding patterns as zymogram were revealed for 79 samples of 23 accessions (Figure 2). The isozyme differences among the landraces of mango from Saptari and Siraha resulted from polymorphism and relative differences in the allelic (band) frequencies for the enzyme EST. Clustering of landraces based on the isozyme banding patterns showed that they could distinctly be identifiable into two genetic groups and showed the band sharing, their frequencies, magnitude of relatedness among the landraces in each tree in the depicted dendrogram (Figure 3). Samples tended to align together in a cluster irrespective of FUDs and the collection sites. Jaccard's similarity coefficients were found for each pair of samples. It ranged from 0.3 to 1.0. Beluwa, Biju, Supariya, Sarahi, Darmi and Motihari had 17–37% intragenetic variation. On the other hand, Bhadaiya and Laduwa accessions segregated into two clusters, indicating a high degree of dissimilarity, although they were called by the same FUDs in different places (Figure 3).

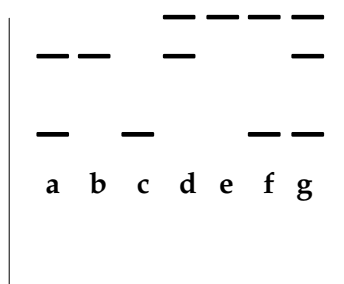


Figure 2. Zymogram of esterase of mango.

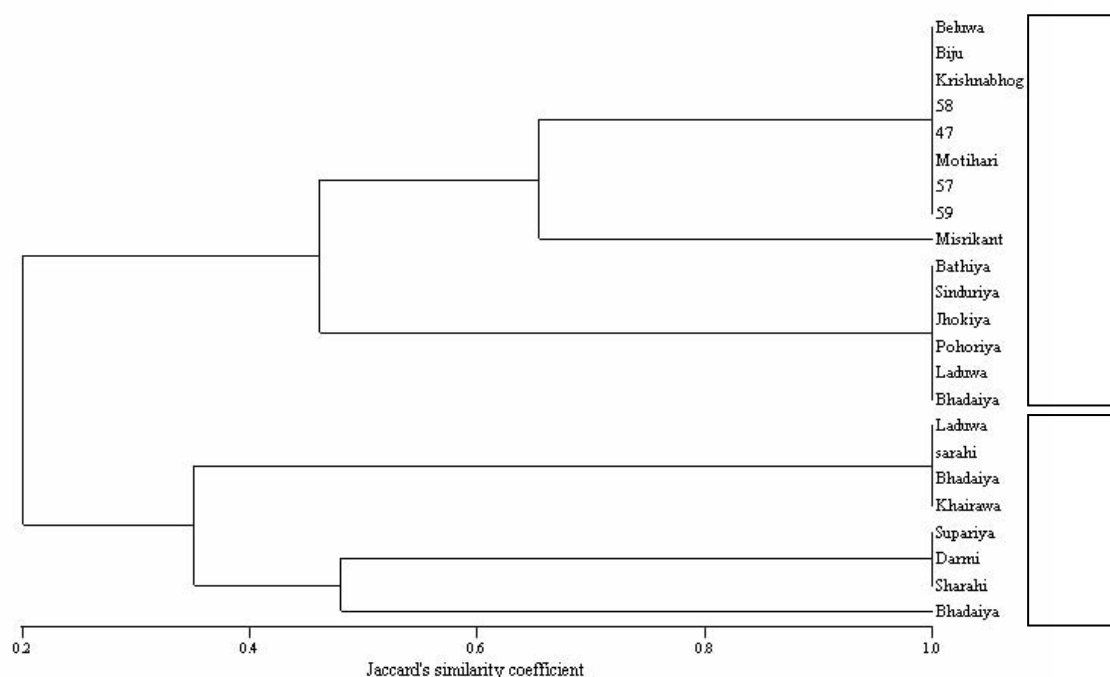


Figure 3. Dendrogram showing relationships between 23 mango landraces based on esterase isozyme allelic diversity. (Boxes are drawn to show the groupings of landraces that fall on clustering using Jaccard's similarity and lines show the 100% similarity).

## Discussion

Although the present study has examined only a few characters of mango fruits, it has found subjectively the existence of a great amount of genetic diversity of mango resources in Nepal but no geographical pattern of distribution was observed. The isozyme study also showed an extent of genetic variation among the landraces with same and different names collected from two adjoining districts. Large variations were revealed in accessions from Eastern and Central regions with high coefficient of variations and high diversity indices. This has demonstrated that quantitative characters are the most influential, acted upon by natural selection under heterogeneous environments of the regions. In contrast, the genetic variability in accessions from the Western region (Parbat and Baglung) was a result of strong selection pressure for desirable fruit qualitative traits under the stress environments of the region.

In fruit tree species, qualitative traits have been found useful in identification and assessment of varieties for fruit production on a large scale as these traits help in developing the ideotypes (Leakey *et al.* 2000). Earlier studies on diversity of crop species within centres

of diversity have demonstrated with examples the importance of quantitative traits outside the centre of diversity and qualitative traits within the centre of diversity (Tolbert *et al.* 1979; Witcombe and Gilani 1979). The accessions from the Western region are less variable than those of the Eastern and Central regions. The large variation in the Eastern and Central regions is limited by natural selection since the observed diversity is of great importance and could affect either the survival or generation of extra diversity.

From the results of this preliminary study on morphological characterization of fruit and biochemical characterization for esterase, a vision is obtained about the level of diversity in mango conserved in farmers' fields of the collection sites. Qualitative and quantitative traits of fruit could be employed in identifying and determining the level of variation. Traditional ways of classifying fruit trees—referring to morphological traits and use values—have conserved the level of genetic diversity. However, further studies with many more enzymes and use of molecular markers need to be continued for efficient use of this diversity.

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## Ecogeographic survey of mango (*Mangifera indica* L.) genetic resources in Nepal

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### Abstract

Mango is one of the important tropical fruits of Nepal. The *terai*, inner *terai*, *siwalik*, low and mid-hills are the regions for rich mango diversity. Home gardens, farmers' orchards, roadside areas, religious or cultural places and river gorge areas are the major areas where both local and commercial varieties of mango are under cultivation or exist as wild species. However, in recent decades many historical mango orchards have been depleted in response to biotic, economic and other pressures. Despite the significant contribution to peoples' livelihoods, no proper assessments of diversity and associated knowledge system have been systematically documented. Also lacking is precise information about the current status of local germplasm or landraces, the location of diversity-rich areas and the ways farmers manage and utilize the crops. Therefore, the global project on "Strengthening scientific basis of *in situ* conservation of agricultural biodiversity on-farm, Nepal component" has identified mango as one of the mandatory crops and under the project, an ecogeographic survey was carried out to measure the extent, distribution and diversities of mango.

Siraha, Dhading, Kavre, Parbat, Baglung, Doti, Surkhet, Banke and Dadeldhura districts, representing the tropical and subtropical ecosystems of Nepal, were selected for study on the basis of ecogeographic representation and richness in mango germplasm. Literature review, a brainstorming meeting, key informant interviews, mango descriptors, ADO consultation and a field visit by a multidisciplinary team were used as methodological tools during the study. Generated data from the study were analyzed and interpreted by Geographical Information System (GIS) software. A customized GIS software for locating and assessing plant diversity—DIVA-GIS (<http://www.diva-gis.org>)—was employed to map collecting sites, analyze the diversity richness and other ecological factors influencing mango diversity. Another customized GIS software—Flora-Map (<http://www.floramap-ciat.org>)—was used to suggest the probability of suitable areas of rich mango diversity for conservation and future exploration. The eastern *terai* represented by Siraha district and western mid-hill region represented by Baglung and Parbat districts were rich in diversity in farmer-named varieties. Cluster analysis has shown three distinct ecogeographical regions: *terai* mango, eastern hill mango and western hill mango. Very old orchards of mango with many indigenous genotypes were recorded from Siraha, Kavre, Parbat and Dadeldhura district and suggested for *in situ* conservation. Throughout the study sites local mango tree trunks were found heavily logged for timber and fuel wood purposes. Low production and high fibre contents in fruits were found as the major factors for the very low commercial production of local mangoes. Lower central and eastern *terai* of Nepal and most of the Indian region and East Asian countries have shown the affinity with Nepalese mangoes and suggested as probable areas for the further distribution of Nepalese mango genotypes.

**Key words:** Ecogeographic survey, genetic diversity, local genotypes, geographic information system (GIS)

## Introduction

Common mango (*Mangifera indica* L.) originated as an allopolyploid and its native home was suggested as Eastern India, Assam to Burma or possibly further in the Malay region (Popenoe 1920). The genus *Mangifera* belongs to the order Sapindales in the family Anacardiaceae with 69 species in the world, mostly restricted to tropical Asia. Mango has rich germplasm diversity and there are about 1600 varieties in the world (Pandey 1998).

Mango is an important tropical fruit of Nepal, having been under cultivation in the country for the last thousand years. Wide ecological variation and diverse needs of multi-ethnic communities have enriched the country with a genetic wealth of diverse mango varieties as landraces. The *terai*, inner *terai*, *Siwalik*, low and mid-hills are the regions of rich mango diversity. Farmers' orchards or home gardens, roadside areas, religious or cultural places and river gorge areas are the major areas where both local and commercial varieties of mango are under cultivation or exist as wild entities. The total area under mango cultivation in the country is 18 114 ha and annual production reached up to 111 400 metric tons (ASD 1999).

Nepalese mango shows a distinct maturing behaviour starting from mid-March to until August onwards. Notable local germplasms include *Sindhure*, *Kali*, *Supare*, *Lohare* from the lower hills and *Chinia*, *Sipiya*, *Chausa*, *Safeda* from the *terai* region; these have the potential for commercial production (NARC 2003). However, in recent years the original habitats of local mangoes have been changed in response to biotic, economic and other pressures. For example, many historical mango orchards have been threatened by logging and a modern agriculture system. Despite the significant contribution to peoples' livelihoods, no proper assessments of diversity and associated knowledge have been documented, nor is there precise information about the current status of local germplasm or landraces; the location of diversity-rich areas and how farmers manage and utilize the crops. The global project on "Strengthening scientific basis of *in situ* conservation of agricultural biodiversity on-farm, Nepal component" identified mango as a mandatory crop and under the project, an ecogeographic survey was carried out to measure the extent, distribution and diversities of mango in Nepal.

## Materials and methods

An ecogeographic study is a process of obtaining, collecting and analyzing different kinds of existing data pertaining to a target taxon within a defined region (Maxted *et al.* 1995). Such studies are validated, refined and complemented by subsequent exploratory field work. The concept of ecogeographic studies was originally developed in the context of conservation of wild plant gene pools and a standardized method applied for crop conservation is still evolving. The ecogeographic survey reported here was carried out following three distinct steps, as illustrated in Figure 1: (1) protocol development, (2) field implementation, and (3) data analysis and reporting.

### Protocol development

#### Thematic group discussion

The thematic group members consisting of social scientists, plant taxonomist *cum* ethnobotanist, agroecologist, germplasm collectors, data analyst and crop biologist from NARC and LI-BIRD thoroughly discussed the objectives, rationale, methods and possible outputs of the mango ecogeographic survey. The broad expertise of the team resulted in the development of a conceptual framework for ecogeographic survey for mango. A draft research protocol consisting of further steps was developed and circulated to *in situ* project professionals and relevant experts of IPGRI for feedback.

**Literature review**

The review was carried out in three steps with specific purposes: first, to understand mango biology, including taxonomy; second, to determine processes and methods currently in practice to carry out an ecogeographic survey. Reports published by IBPGR (1985), Maxted *et al.* (1995) and Guarino (1995) were consulted to understand the mango description and methodology for survey. The existing situation of mango genetic diversity in Nepal has been reviewed based on the reports available in libraries from NARC, DoA, RONAST, ICIMOD, TU, IPGRI-APO and grey literature. The survey sites were finally selected on the basis of diversity richness and ecogeographic representation.

**Consultation workshop with national crop experts**

A draft research protocol was shared with national experts through an organized consultation workshop, where the protocol was further refined and the target sites (district) for the survey were selected. These workshop outcomes also were shared with the thematic members for further refinement and necessary preparation for field work.

**Field implementation****Multidisciplinary team and subteam set up for survey**

A team consisting of plant taxonomist *cum* ethnobotanist, social scientist, germplasm collectors, crop biologist and support staff members carried out the survey; three different subteams were established to facilitate the survey work in *terai* and mid-hill ecosystems of Nepal. GPS was used to record the coordinate values and altitudes.

**Checklist preparation**

A checklist consisting of relevant information to be documented was prepared following a review of mango descriptors (IPGRI 1989) and thematic group discussion (Table 1).

**Knowledge on GIS methodology**

Members involved in the ecogeographic survey participated in a training programme on GIS application for plant genetic resources conservation provided by IPGRI-APO. This training provided information about the use of customized GIS softwares—DIVA-GIS (<http://www.diva-gis.org>) and Flora Map (<http://www.floramap-ciat.org>)—relevant for mapping, locating and assessing crop diversity.

**Pre-test of survey**

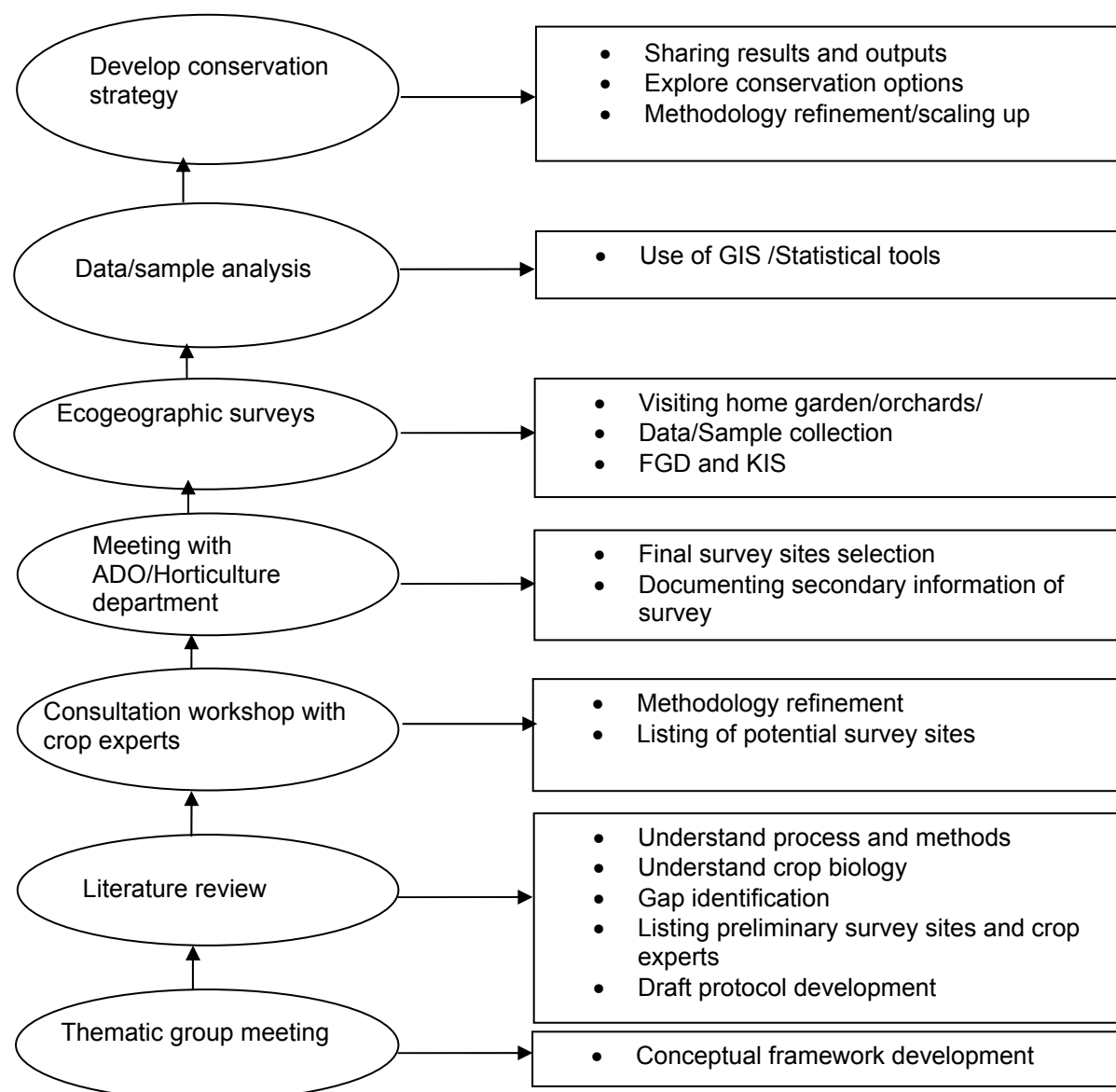
To develop a common understanding on survey methodology, a pre-test programme was organized at one site, where all members of the survey team participated.

**Consultation with ADO and horticulture divisions of survey districts**

Meetings with ADO and scientists of the Horticulture Division of Department of Agriculture (DoA) at each of the survey districts were organized to get in-depth information about mango-growing rich areas, production rate, varieties' status, rainfall pattern, soil characteristics and major problems faced by the mango farmers. During discussion a list of key resource farmers of each selected sites was prepared.

**Survey and documentation**

The team visited at least 3–7 target sites within each district. The target sites represented the farmers' mango orchards, home gardens, demonstration blocks of the Horticulture Division or local weekly markets (*hatiya*). During the survey, key informants were noted for future local knowledge acquisition on mango.



**Figure 1. Process, steps and methods employed for the ecogeographic survey of mango in Nepal.**

### Characterization of local mango genotypes

Local mango genotypes were differentiated from the commercial and hybrid based on the information provided by key resource farmers and technical staff. Similarly, relevant literature—IBPGR Mango descriptors and internet sources (<http://www.horticultureworld.net>)—were used to further validate the information.

**Table 1. Checklist of mango varieties collected from different survey sites of Nepal.**

Region	District(s)	Location(s)	Alt. (m asl)	Farmer-named varieties (genotypes)
Eastern	Siraha	Bishnupur	100	Amarpali, Alfanso, Barbariya local, Bathuwa, Bhonth, Bhadaiya, Darmi, Fajali, Jarmale, Kalkatia, Karelwa, Kapuriya, Keruwa, Krishnabhog, Krishnabhog biju 1,2,3 and 4, Bombay, Lakhnau Bombay, Nawaras Bombay, Neelam, Chiniya, Bombay biju 1 and 2, Laduwa, Maldhua, Malika, Mishrik, Radhi, Sindhuriya, Sipiya, Sridhanka, Supariya, Suryapuri (36)
		Mirchaiya, Ramnagar-9	100	
		Lahan, Lahan-4	125	
	Saptari	Kanchanpur-6	130	
		Kalyanpur-5	140	
		Jandole-6	90	
		Rajdevi, Rajbiraj-9	150	
	Dhanusa	Devpuri-rupani-2	90	
		Devpuri-rupani-3	90	
		Kuwa, Janakpur-12	90	
		Ghodghans-1	90	
		Ghodghans-2	90	
Central	Kavre	Aanpghari, Baluwa –3		Banarasi, Bellure, Bhadaure, Biju-1, 2, 3, Bombay, Bomabay green, Chucho kali, Diyale, Dhupi, Dhobi kali, Jhuttare, Kali, Kali biju, Kere, Kari, Kakre, Labate, Lohare, Lokharke, Maldawa, Malta-Biju, Mishree, Pharse, Saune, Saune malta, Saune maldawa, Sindhure, Sindhure kali, Supare, Thulo kali, (33)
		Balakhe, Shikharpur-1		
		Jadetar, Baluwa-9		
		Kharkachowr-Baluwa-9		
		Bagaicha, Kharketar-6		
	Dhading	Baireni, Malang –7	630	
		Baharbote, Nalang-6	630	
		Muralibhanjyang-3	620	
		Majhitar, Kumpur-3	580	
		Sayale, Khanikhola-1	450	
		Seplaji, Khanikhola-9	1020	
		Badritar, Jibanpur-8	1000	
	Parbat	Dimuwa, Tilahar-6	850	Bahure, Bhatte, Bhettne, Bombay, Chope, Dahe, Diyale, Dudule, Dum Gande, Genaurre, Harre, Hade, Jirre, Kamile, Kawale, Kera pake, Koye, Lamadaya, Lamche, Mitthe, Naite, Pani aanp, Patali, Rato kupu, Rato chake, Sano kamile, Seti Bombay, Supare (28)
		Saharshadhara, Pang-1	730	
		Pang-2	900	
		Patedhunga, Mudkuwa-5	820	
		Dharmasala,	830	
		Devisthan-9		
		Rale, Devisthan-8	830	
		Ekghare, Mudkuwa-1	820	
Western	Baglung	Baglung Municipality-1	920	Aamrapali, Bombay, Dalle, Dashari, Gitthe, Kali, Lohare, Mitthe, Naite, Supare (10)
		Kalika mindir, Baglung	920	
		Niraye, Baglung Municipality-11	760	

**Table 1 (cont.)**

Region	District(s)	Location(s)	Alt. (m asl)	Farmer-named varieties (genotypes)
Far Western	Dadeldhura	Samaiji-1	1430	Achare local, Bombay, Bombay Kathe,
		Samaiji-2	1400	Bombay-green, Chaksa, Dasahari, Dasahari
		Mastamandu-3		biju, Fajeri, Langda, Langdi-biju, Local biju, Local golakar biju, Local Kathya, Local-1,2,3,4, Rithya
	Banke	Bhauniyapur- 5 and 7	130	Gola, , Kapuri, Lakhnaw –safeda, Sinduriya
		Suryapur, Udaipur-5	150	
	Surkhet	Chhinchu-7	540	Bombay, Chausa, Dasahari, Kalkatiya, Local
		Ramghat-6	490	Dasahari biju, Safeda
		Birendranagar	590	
	Doti	Punnagaun, Silgadi –5	1490	Bombay, Bombay-green, Dasahari, Dasahari,
		Sungada,Dipayal-7 and 8	650	Dhaulya, Fajali, Fajari, Kalya, Kokya, Langda, Local (Kathi), Maldaha
		Talkot, Pachnali-6	560	
		Bandumrisain, Banlekh-4	620	

## Data analysis and reporting

### Roundup meeting of survey teams

Before analyzing the data, a roundup meeting was organized and each subteam highlighted their major findings and shared the problems and constraints encountered during the survey with other team members.

### Analysis

All standardized data were entered into a computer using Microsoft Excel and survey analysis was carried out using DIVA-GIS to analyze the diversity and richness analysis, and Flora Map to predict the distribution of diversity rich-areas at sites for introduction of suitable mango landraces.

## Results and discussion

### Collecting mango germplasm

The survey sites (Figure 2) are in diverse geography covering the Gangetic Indian plain, inner *terai*, inner valleys and river gorge areas of Nepal with wide altitudinal variation ranging from 90 to 1500 m asl. A total of 32 ecogeographic surveys were undertaken at the target areas of the districts: Siraha, Saptari, Dhanusa, Banke (*terai* districts) and Kavre, Dhading, Parbat, Baglung, Surkhet, Doti, Dadeldhura (mid-hill districts). Information and accessions of mango genotypes were collected with the help of local farmers. In the process, due consideration was also given to visiting old mango orchards grown by communities or existing as wild entities.

### Extent and distribution of mango diversity

A total of 899 accessions of mango were collected from the target sites. Owing to wide geographical representations, a diverse genetic diversity of mango varieties with both commercial and local genotypes were documented and evaluated on the basis of IPGRI descriptors (1989) and farmers' existing knowledge. The study was able to record a total of 132 farmer-named varieties of mango from the survey sites (Table 2). Among them, a rich diversity was recorded from Siraha, Saptari, Dhading, Kavre, Parbat and Dadeldhura districts representing a total of 62, 43, 48, 31, 29 and 21 farmer-named varieties, respectively.

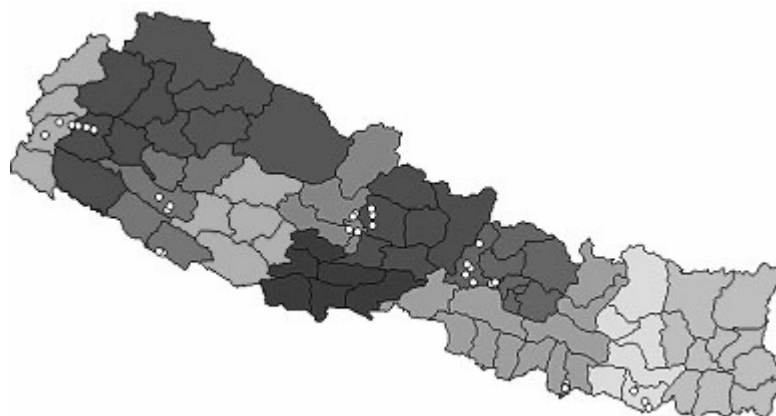


Figure 2. Collecting sites of mango in Nepal.

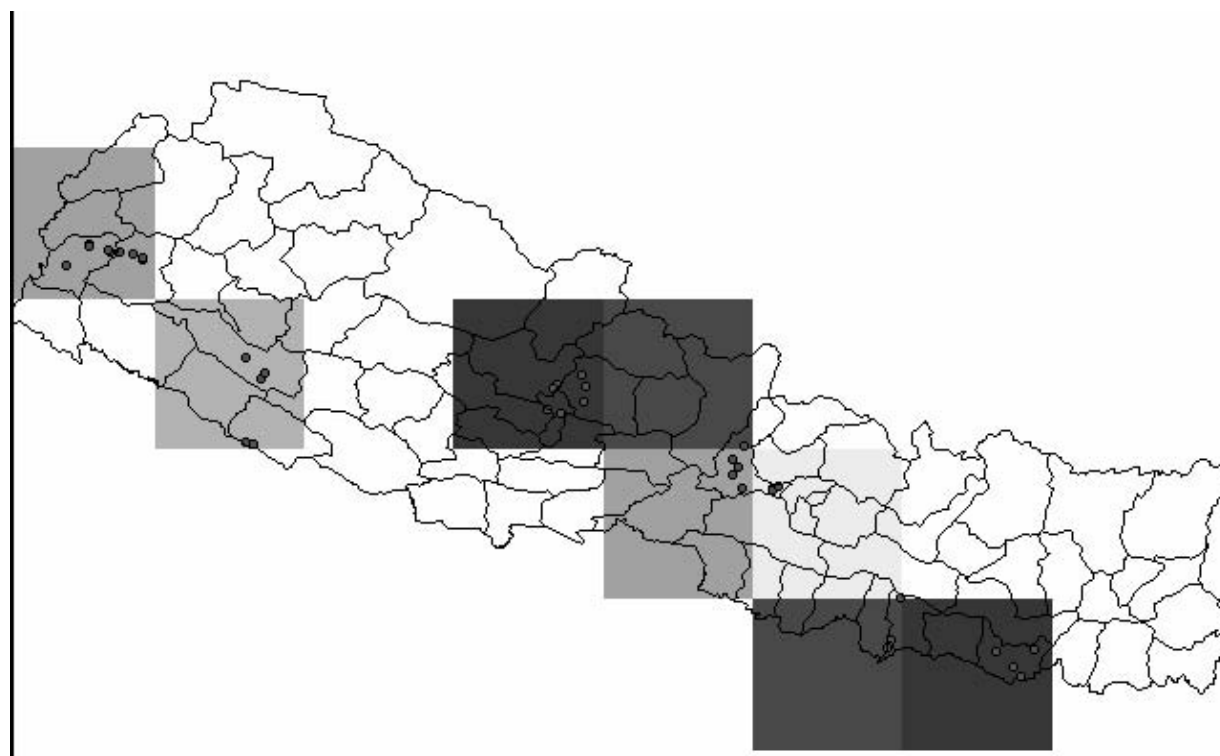
Table 2. Possible *in situ* conservation sites for mango genetic resources in Nepal.

Ecosite	Ecogeographical characteristics	Total varieties	Unique local genotypes
<b>Samijil</b> , Samijil VDC, Dadeldhura district (Far-western Nepal)	Middle mountain with valley bottoms (1400-1430 m asl.)	21	Achare, Kathe, Kathya and Rithya
<b>Pang</b> , Pang VDC, Parbat district (Mid-western Nepal)	Middle mountain (900 m asl)	25	Bahure, Bhetne, Bhatte, Chope, Dayhe, Diyale, Doom Harre, Kamile, Lamche, Naite, Rato-kupu, Patali and Supare
<b>Majhitar</b> , Kumpur VDC and <b>Badritar</b> Jibanpur VDC, Dhading district (Western Nepal)	Middle mountain with river valleys (550-1000 m asl)	33	Alini, Aama-bubu, Boke tauke, Budhi, Hade, Jhutte, Kali, Lohare, Supare, Jwane, Seti, Saune
<b>Bagaichya</b> , Kharketar-6, Kavre District (Central Nepal)		30	Bellure, Chucho kali, Diyale, Dhupi, Dhobi kali, Jhuttire, Kere, Kari, Kakre, Labate, Lohare, Lokharke, Supare and Thulo kali
<b>Mirchiya</b> , Ramnagar -9, Siraha district (Eastern Nepal)	Indo-Gangetic plain with river bottom (100 m asl)	35	Barbariya, Bathuwa, Bhonth, Darmi, Jarmale, Karelwa, Keruwa, Laduwa, Sridhanka, Supariy,

### Locating diversity-rich areas of mango

Although distribution of mango genotypes was scattered throughout the *terai* plain and mid-hills of survey sites (Figure 3), a few ecogeographic sites were found unique owing to the existence of a high number of farmer-named diversity, with dominance of local genotypes, covering a large area with old (>150 years) mango orchards. These sites also represent the diverse ecogeography and social-cultural environments.

Many of the varieties recorded from these sites were found to be unique in their local name, shape, size and maturing behaviour. Therefore, these sites were suggested as *in situ* conservation sites of mango genetic resources in Nepal.



**Figure 3. Diversity-rich areas of mango genetic resources in Nepal based on local name.**

### Locating local mango genotypes with economic potential

The existing market situation has shown that only a few genotypes occupy the major portion of the mango industry within the country. These varieties are mostly Indian varieties like *Bombay*, *Bombay Green*, *Chausa*, *Dashehari*, *Fazli*, *Kishen Bhog*, *Langra*, *Neelum* and *Chausa*, and hybrid varieties. Local mango genotypes from the survey sites that retained commercial potential were documented (Table 3). These local mangoes have been used as fruits, or for local use purposes such as pickle, salad or juice.

**Table 3. Local mango genotypes with potential commercial value.**

Fruit value	Juice value	Pickle and salad value
Chinia	Harre	Achare
Chuche kali	Kalya	Bhatte
Chulesi	Kathe	Gande
Kalame	Kerapake	Githhe
Kalapahad	Koke	Gola
Kali	Pani aanp	Jarmale
Lamdaya	Rato tauke	Jhutte
Lohare	Supare	Jhuttre
Mithhe		Rithya
Naite		Thulo kamile
Safeda		
Sindhure		
Sipiya		
Pharse		
Tamburiya		
Thulo kali		



### Probable distribution of diversity of mango in Nepal and Asia

The Flora Map was used to map the distribution of diversity of mango genetic resources (Figures 4 and 5). Lower central and eastern *terai* regions of Nepal and most of the Indian region and East Asian countries have shown an affinity with Nepalese mangoes.

### Existing threat to local mango genetic resources

Mango orchards of survey districts such as Siraha, Saptari and Kavre have been largely threatened by logging and the modern agriculture system. Local mango trees have been excessively used in brick factories, the furniture industry and for fuel wood purposes. And, there is an increasing trend of cultivation of hybrid or improved varieties of mango among the farmers throughout the survey sites. This case is more severe in *terai* region of Nepal because of easy access to improved genotypes. This has resulted in genetic erosion of local mango genotypes as well as loss of potential local varieties. The key informant interviews showed that many of the local mango genotypes were not preferred in the market in comparison with modern varieties because of the high fiber content of the flesh, large size of seed and delay in fruit-bearing behaviour. However, this was not always completely true and several local mango genotypes were recorded during the survey periods that have retained high commercial value.

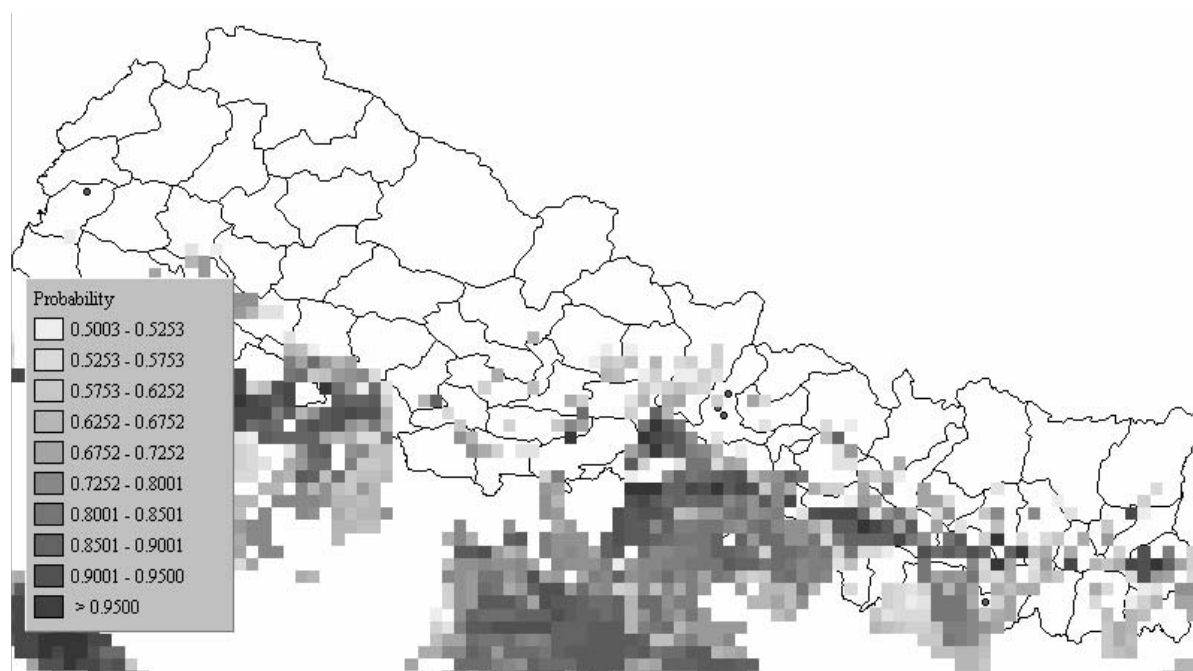
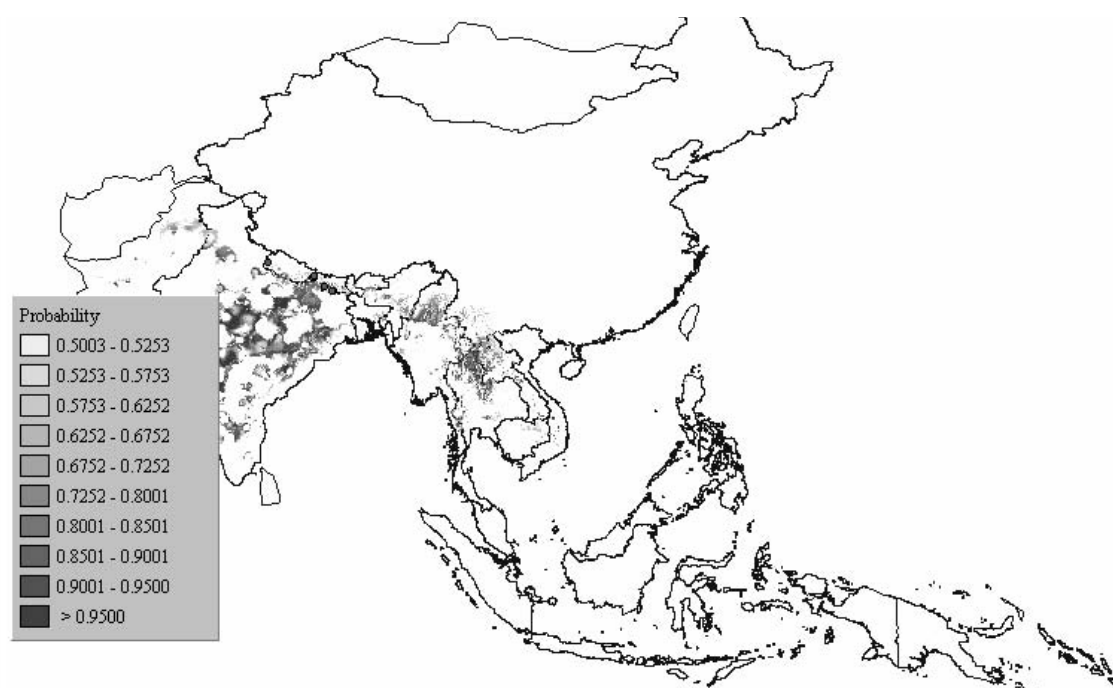


Figure 4. Probability map of mango genotypes distribution in Nepal.

### Conclusion

Ecogeographic surveys in target districts have revealed the existence of wide genetic diversity in mango genetic resources in Nepal. A total of 899 accessions of mango were collected from the target sites and diversity was mapped. The study was able to record 132 farmer-named varieties of mango from the survey sites. A rich diversity was recorded from Siraha, Saptari, Dhading, Kavre, Parbat and Dadeldhura districts representing a total of 62, 43, 48, 31, 29 and 21 farmer-named varieties, respectively. A few target sites were found unique in diversity, which were therefore suggested for *in situ* conservation sites of mango genetic resources. Similarly, potential local genotypes with commercial values were



**Figure 5. Probable distribution of Nepalese mango genotypes in Asia.**

identified from the survey sites and highlighted. A total of 12 flora maps were produced and pictures were digitized. However, in recent years the original habitats of local mangoes have been changed in response to biotic, economic and other pressures. Therefore, promotion of local genotypes at farmer orchards and detailed study in identified rich areas for mango were suggested for the conservation and sustainable utilization of mango genetic resources in Nepal.

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*Note:* IPGRI = International Plant Genetic Resources Institute; LI-BIRD = Local Initiatives for Biodiversity, Research and Development; NARC = Nepal Agricultural Research Council.

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