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Variation in rice landraces adapted to the lowlands and hills in Nepal

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Abstract

The landraces of rice (*Oryza sativa* L.) possess wide diversity, which needs to be properly characterized for their use in genetic improvement. Replicated field studies were conducted in 1998, 1999 and 2000 at two sites in Nepal to determine diversity in 183 landraces of rice adapted to the lowlands and the hills in Nepal. Fourteen improved genotypes were also used for comparison. Thirteen agronomic traits were investigated. Shannon–Weaver diversity index (*H*) and Simpson's index of diversity (*D*) were estimated to determine the level of genetic richness among the landraces. The landraces differed significantly for all traits. Except for plant height and maturity, at least one of the landraces compared well with the performance of improved cultivars. A principal component analysis separated the lowland- and hill-adapted landraces into two broad groups.

Keywords: genetic diversity; landraces; Nepal; *Oryza sativa*; rice

Introduction

Rice (*Oryza sativa* L.) is cultivated across diverse agroclimatic environments in five out of the six continents (Lu and Chang, 1980). Rice genotypes are classified primarily into the subspecies *indica* and *japonica* (Oka, 1958). The *indica* type comprises primarily tropical lowland genotypes, while the vast majority of cultivars grown in temperate regions are of the *japonica* type. The *japonica* type is also cultivated in high elevation and dryland areas of the tropics (Glaszmann and Arraudeau, 1986).

The agroecosystem in Nepal is diverse because altitude ranges from 60 m above sea level (asl) in the southern plains to above 8800 m asl in the Himalaya, within a north–south stretch of <230 km. The country is broadly divided into lowlands, foothills including valleys, midhills, high hills and mountains. Rice is grown in flatland, hills and high mountains from 60 to 3000 m asl (Whiteman, 1985; Nagamine, 1992; Gauchan *et al.*, 2003; Bajracharya *et al.*, 2006). Diverse and agronomically useful wild rice genotypes have been found (Suh *et al.*, 1997; Lu, 1999), and the diversity of Nepalese rice is reflected by a large collection of landraces and several wild rice species (Gauchan *et al.*, 2003; IRRI, 2005). However, passport data for these landraces are limited, and as a result, they have contributed little to national and international rice improvement. However, many landraces are still grown commercially for their traditional uses and desirable characters (Chaudhary *et al.*, 2004). Premium prices can be paid for special traits such as fineness of grain, cooking quality, taste and aroma (Gauchan *et al.*, 2005). A few landraces show tolerance to water-submergence and chilling (Sthapit *et al.*, 1995; Sthapit and Witcombe, 1998), as well as disease resistance (Karki, 1989; Sthapit *et al.*, 1995).

In some areas a combination of landraces and modern rice cultivars is grown, whereas in certain remote districts, exclusively landraces are cultivated (Gauchan *et al.*, 2005). The success in breeding improved rice cultivars since the 1960 s has helped increase rice production, but has led to the replacement of a large number of landraces by a small number of improved cultivars. Thus a gradual but continuous loss of genetic diversity is affecting the viability of rice landraces in

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Nepal (Chaudhary *et al*., 2004). Maintaining rice landrace diversity and utilizing it for genetic improvement is a major thrust in current rice improvement activities (Joshi and Witcombe, 2003). A prerequisite for this is an understanding of the diversity present in these landraces, and we describe aspects of this in the present paper.

Materials and methods

A set of 197 rice genotypes was used in this study [Supplementary Table 1 (available online only at http://journals.cambridge.org)]. This included 110 lowland and 73 upland landraces and 14 improved rice genotypes bred in Nepal, Malaysia, Vietnam and the Philippines, including the widely adapted IRRI-bred IR64 and IR72. Although characterized as 'upland' or 'lowland', based on site of collection, many of the landraces are also grown in the valleys and foothills, in a transition zone between the hills and the plains.

Landrace seed was obtained from the Agriculture Botany Division, Nepal Agricultural Research Council (NARC), Khumaltar, and other materials were obtained from the International Rice Research Institute (IRRI), Philippines. Rice landraces are documented by accession number, but a few entries share the same or similar names. In the past, the farmers in the remote areas have also given their own vernacular names to a few rice landraces that sound like another crop, as is the case for genotypes 91 to 110 (Supplementary Table 1).

Field trials were conducted at Rampur (Chitwan district) and at Paklihawa (Rupandehi district) in the 1998, 1999 and 2000 main rice seasons. The former site is at 228 m asl, in the Himalayan foothills on a medium-textured loam, and the latter a heavy clay at 105 m asl in the outer plains. The trial was conducted in a randomized complete block with three replicates. The size of each experimental plot was 1.8×2.4 m, transplanted with 25-day-old seedlings at 15×15 cm spacing. Fertilizers were applied prior to transplanting at the rate of 80, 60 and 40 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. The plots were kept free of weeds by hand weeding, and were rainfed, except where initial irrigation was required at transplanting.

The following traits were recorded: days to heading (DH) and maturity (DM), plant height (PHT), effective number of tillers per unit area (ETN), panicle length (PL), percent of unfilled grains (PUG), thousand-grain weight (TGW), biomass yield (BY), grain yield (GY) and colour of mature grains, following the guidelines specified by IRRI (1996). DH was taken when approximately 50% of tillers in a plot had panicles fully emerged, and DM when the grains had reached physiological maturity. The duration of grain-filling (DGF) was determined by subtracting DH from DM. PHT was the height as measured from the ground level to the tip of the panicles. The number of panicles per plant (PPP) was determined by dividing ETN by the number of plants per unit area. PL was determined as the mean of ten randomly chosen panicles, which were also used to determine PUG. At harvest, plots were cut close to the ground, and bundles from each plot were dried in a glasshouse for several days. These bundles were used for the determination of BY and GY. Straw yield (STY) was the difference between GY from BY. Harvest index (HI) was given by 100 $\times$ the ratio GY/BY. To obtain TGW, 1000 grains were counted and weighed from the harvest of each plot.

Analyses of variance were computed for each site in each year to estimate the contribution of genotype to overall variation. Pairwise correlation coefficients (r) among six site–year combinations were determined to assess consistency in expression. Average simple correlation coefficients (r_S) over locations and years were calculated using Fisher's z-transformation, as outlined by Steel and Torrie (1980). Genotypic correlations (r_G) among various traits were determined using the procedure outlined by Falconer (1981). All statistical analyses were conducted using MINITAB software (MINITAB, 2000).

The level of diversity among landraces was described by the Shannon–Weaver diversity index (H) and Simpson's index of diversity (D) using the procedure outlined by Chaudhary *et al.* (2004). A multivariate analysis using all 13 traits was made using SAS (2003) software to cluster the accessions and produce a graphical representation of the diversity present.

Results

The combined analysis of variance showed that the effects of year and location were significant for all traits [Supplementary Table 2 (available online only at http://journals.cambridge.org)]. The year-by-location interaction was significant for BY, STY, GY and HI. The genotypic effect was significant for all 13 traits. Genotype-by-location and genotype-by-year interactions were non-significant for all traits, whereas genotype-by-location-by-year interaction was significant for all traits.

The 197 rice genotypes showed a range of variation for all characters [Supplementary Table 3 (available online only at http://journals.cambridge.org)]. A comparative analysis of three groups of genotypes, lowland and hill-adapted landraces and improved genotypes, is shown in Table 1. On average, the lowland-adapted landraces were later in heading and maturity by 20 and 25 days, respectively, compared to the mean of the improved genotypes. The longest DGF among the lowland adapted group was six days more (46 days, #31) than the improved genotype (40 days, #195) (Supplementary Table 3). In general, the earliest heading landraces did not necessarily have a longer grain-filling period. Commonly, the landraces were taller than the improved genotypes; however, the lowland-adapted landrace with the shortest height was only 4 cm taller (90 cm, #86) than the improved genotype (86 cm, #185). The lowland-adapted and improved genotype groups did not differ significantly in average values for effective tiller number and panicle per plant (Table 1). However, the lowland-adapted landrace with the highest ETN produced 17% more tillers (259, #87) than the improved genotype (222, #184). Number of tillers per plant is an important trait in developing appropriate plant type for a specific rice ecosystem. There was no significant difference in mean panicle length, thousand-grain weight and percent unfilled grain among the three groups (Table 1). However, the range of variation was wider for these traits among the landraces than the improved genotypes. Average BY and STY were higher for the lowland-adapted group than for improved genotypes, whereas mean GY of the two groups was almost equal.

Most of the landraces were photoperiod sensitive. This is reflected in the comparative frequency distribution of the two groups of landraces (Fig. 1A and B). Despite late heading, the lowland-adapted landraces, in general, spent a longer period in grain filling than the hill-adapted landraces (Fig. 1C).

There was no trend for the two groups of landraces for plant height (Fig. 1D). Plant heights of a number landraces compared well with the height of some of the improved genotypes but were taller than IR72. There was no particular trend in effective tiller number and the number of panicles per plant in the two groups of landraces (Figs 1E and F). Panicle lengths of the two groups of landraces were randomly distributed without a particular trend (Fig. 2A). Thousand-grain-weight was randomly distributed between the two groups of landraces (Fig. 2B). Despite a normal distribution of percent unfilled grain of the landraces, a relatively larger number of lowland-adapted landraces showed a higher value for this trait (Fig. 2C). In general, there was a low percentage of unfilled grain among the improved genotypes than the landraces.

A large number of the lowland-adapted landraces showed high BY, STY and GY (Fig. 2D, E and F). The improved genotypes showed low to intermediate BY and STY but intermediate to high GY. Harvest index values of the two groups of landraces were normally distributed (Fig. 2G).

The landraces exhibited nine distinct shades of colour (Supplementary Table 3). The two important characters of

Table 1. Range for various characters in three groups of rice genotypes grown at two locations in Nepal from 1998 to 2000

Source	Genotype group	DH	DM	DGF days	PHT cm	ETN m^{-2}	PPP $plant^{-1}$	PL cm	TGW g	PUG %	BY $t\ ha^{-1}$	STY $t\ ha^{-1}$	GY $t\ ha^{-1}$	HI %
Mean	$LR_{Lowland}$[a]	119	157	38	145	184	6.2	25.4	22.3	11.5	10.690	7.666	2.932	28.1
	LR_{Hills}	91	123	31	145	144	4.9	26.3	21.5	10.1	7.081	4.636	2.238	32.1
	IG	99	132	33	115	177	6.0	25.3	20.2	10.1	8.351	5.083	2.964	35.8
Minimum	$LR_{Lowland}$	80	114	26	90	78	3.2	19.6	12.0	7.8	4.591	2.658	1.107	16.4
	LR_{Hills}	79	113	25	120	81	2.6	22.4	14.3	7.1	4.229	2.799	1.078	18.9
	IG	85	119	27	86	134	4.6	22.1	15.8	7.9	5.098	2.997	1.871	29.4
Maximum	$LR_{Lowland}$	135	174	46	165	259	9.5	34.1	30.1	17.1	15.159	10.708	4.712	46.4
	LR_{Hills}	112	142	40	172	257	8.9	32.7	30.2	14.0	10.944	7.636	3.520	41.5
	IG	112	145	40	139	222	7.9	31.2	26.4	16.3	12.264	7.397	4.489	43.9
H[b]	$LR_{Lowland}$	0.49	0.48	0.52	0.46	0.59	0.57	0.49	0.58	0.56	0.54	0.53	0.56	0.54
	LR_{Hills}	0.43	0.30	0.37	0.38	0.51	0.50	0.58	0.45	0.45	0.39	0.42	0.42	0.41
D[c]	$LR_{Lowland}$	0.61	0.60	0.65	0.58	0.71	0.70	0.62	0.72	0.70	0.69	0.68	0.70	0.67
	LR_{Hills}	0.60	0.50	0.52	0.54	0.65	0.64	0.73	0.63	0.62	0.56	0.59	0.57	0.58

DH, days to heading; DM, days to maturity; DGF, duration of grain filling; PHT, plant height; ETN, effective tiller number; PPP, number of panicles per plant; PL, panicle length; TGW, thousand grain weight; PUG, percent of unfilled grains; BY, biomass yield; STY, straw yield; GY, grain yield; HI, harvest index.
[a] $LR_{Lowland}$, landraces adapted to lowlands; LR_{Hills}, landraces adapted to hills; IG, improved genotypes.
[b] *H*, Shannon–Weaver diversity index.
[c] *D*, Simpson index of diversity.

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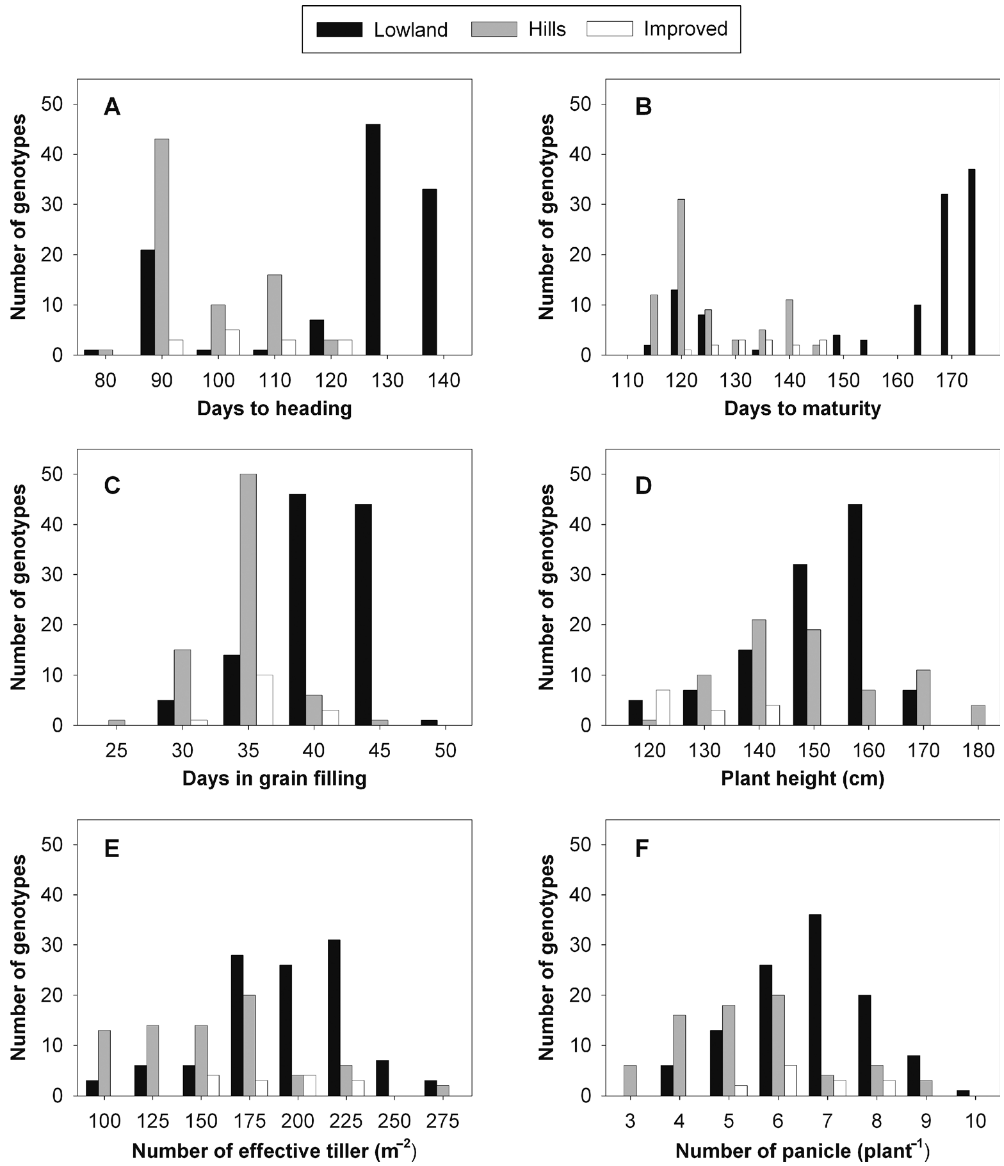

Fig. 1. Frequency distribution for days to heading (A) and maturity (B), days in grain filling period (C), plant height (D), number of effective tillers (E) and number of panicles (F) in 110 lowland- and 73 hill-adapted landraces and 14 improved genotypes of rice grown at Rampur and Paklihawa, Nepal, in the main rice season from 1998 to 2000.

economic importance, GY and STY, showed significant positive simple ($r_S = 0.603$, $P = 0.01$) [Supplementary Table 4 (available online only at http://journals.cambridge.org)] and genotypic ($r_G = 0.39$) [Supplementary Table 5 (available online only at http://journals.cambridge.org)] correlations. There was a high positive simple ($r_S = 0.774$, $P = 0.01$) (Supplementary Table 4) and genotypic ($r_G = 0.60$) correlation coefficients (Supplementary Table 5) between BY and GY.

A range of values was obtained for the two indices *H* and *D* used to measure the diversity within each group of germplasm (Table 1). The *H* value ranged from 0.46 to 0.59 and from 0.30 to 0.58 for the lowland and the hill groups, respectively. The *D* value ranged from 0.58 to 0.72 and from 0.50 to 0.73 for the lowland and the hill groups, respectively. The *H* value for each trait was higher for the lowland- compared to the hill-adapted landraces. The *D* value for individual traits among the lowland landraces was either higher than or equal to the corresponding values among the hill-adapted genotypes. The paired *t*-test showed a significant ($P < 0.01$) difference in genetic diversity of the two groups of landraces considering all 13 traits examined in this study.

A principal component analysis based on 13 quantitative traits showed two broad categories representing

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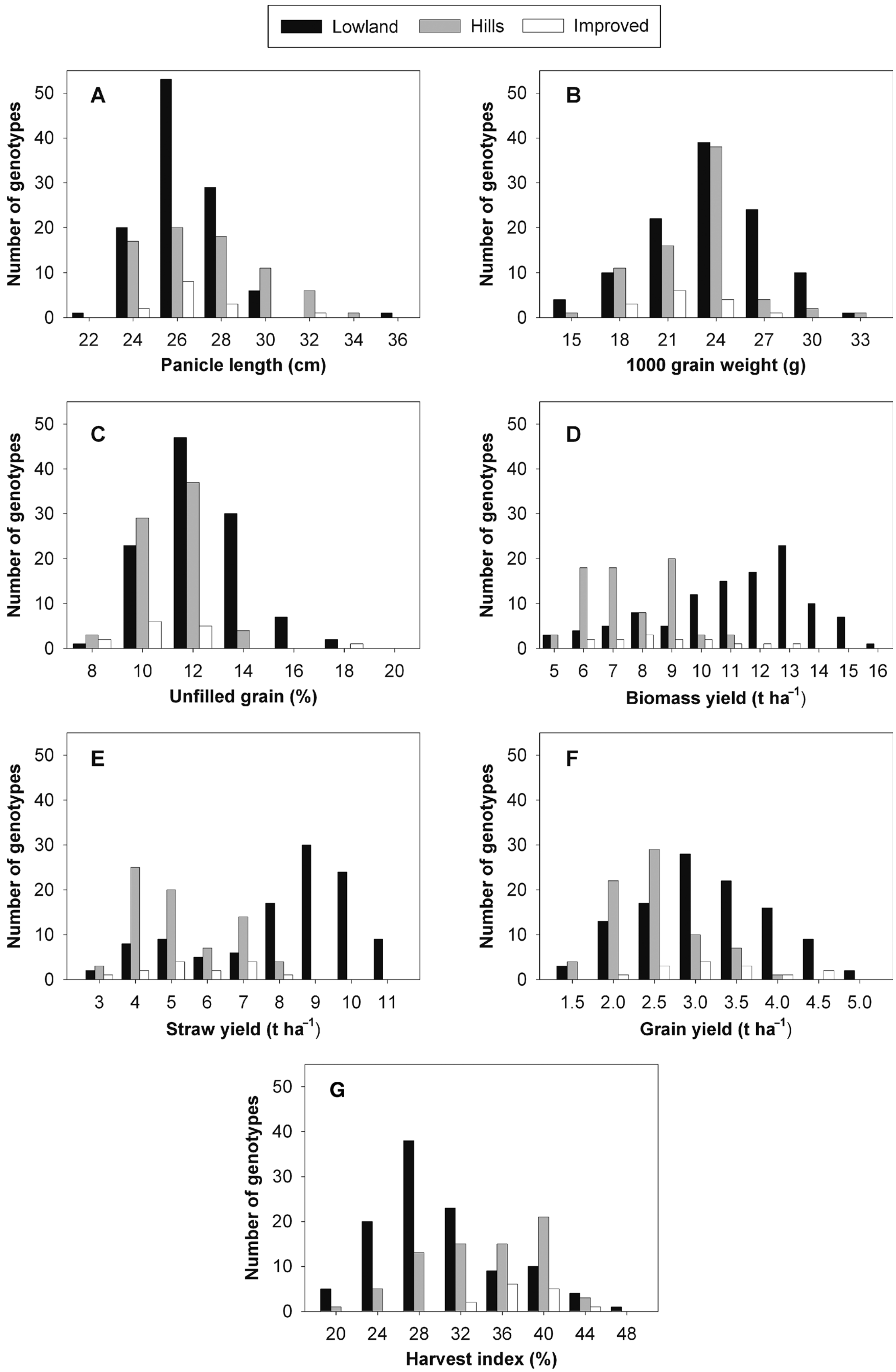

Fig. 2. Frequency distribution for panicle length (A), thousand grain weight (B), unfilled grain (C), biomass yield (D), straw yield (E), grain yield (F) and harvest index (G) in 110 lowland- and 73 hill-adapted landraces and 14 improved genotypes of rice grown at Rampur and Paklihawa, Nepal, in the main rice season from 1998 to 2000.

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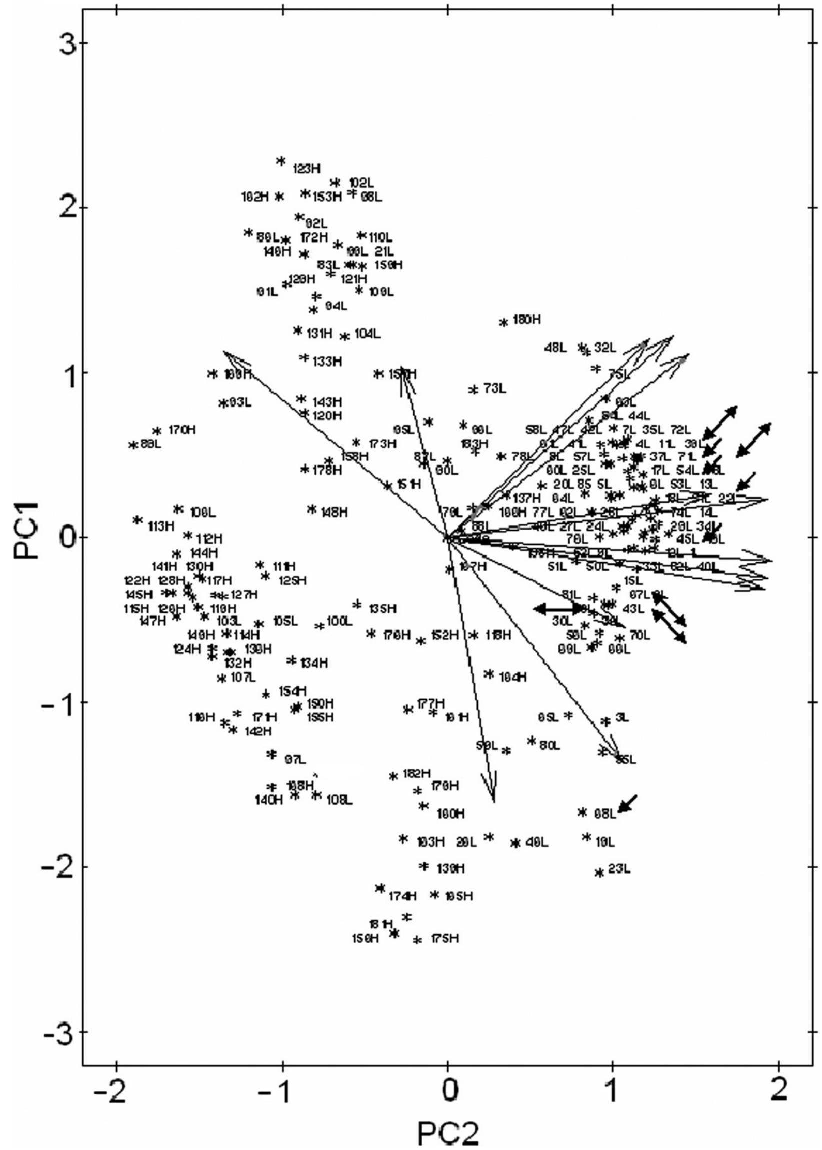

Fig. 3. Multivariate analysis showing principal components biplot of 110 lowland- and 73 hill-adapted rice landraces in Nepal based on 13 phenotypic traits evaluated at two sites in 1998, 1999 and 2000 main rice seasons. (The numbers in the body of the biplot correspond to entry number from Supplementary Table 1, and H and L symbolize hill- and lowland-adapted landraces, respectively). (PC1 and PC2 contributed 65% of total variation).

the lowland- and the hill-adapted landraces with few exceptions (Fig. 3). Nonetheless, there was a continuum of diversity among the studied landrace populations. Also, there were a few genotypes in each group that clustered with the landraces of another group. For example, the lowland-adapted genotypes #103 and #107 clustered closely with the hill group. On the other hand, hill genotypes #137 and #166 grouped with the lowland-adapted landraces. Within each set of landraces, there were secondary clusters indicating intra-group diversity.

Several landraces had the same name; however, they didn't always group together. For example, five landraces (#22, #45, #54, #68 and #71) had the same farmers' given name, 'Bakai' (Supplementary Table 1). These landraces are marked by an arrow sign on the biplot (Fig. 3). The landraces, #54 and #71 grouped together tightly, #22 was a little separated, #45 was further separated, and #68 was separated far out of the cluster. Similarly, another set of five lowland-adapted landraces had the identical name, 'Basmati' (#10, #38, #39, #43 and #67) (Supplementary Table 1). These landraces are marked by a double arrow sign on the biplot (Fig. 3). Among these five landraces, #38 and #39 clustered together, separated from another group that included #10, #43 and #67. Similar results were found for other landraces with identical names.

Discussion

The rice growing conditions were typical for the area at both locations in all 3 years; however, there was some year-to-year variation. Rainfall during early stages of crop growth, after transplanting (July and August), was sufficient in 3 years at both locations [Supplementary Fig. 1 (available online only at http://journals.cambridge.org)]. The total rainfall for the rice-growing season was the highest in 1998 and the lowest in 2000 at both sites. The results show that relative differences between locations differed significantly in the 3 years. Also, genotype-by-location interactions differed in the 3 years.

The arrays of genetic variation among landraces suggest that these could be used in improving the agronomic and yield traits (Supplementary Table 3). The longer grain filling period of the lowland-adapted landraces than that of hill-adapted genotypes offers potential for expanding the grain filling period and increasing grain yield by combining early heading with normal maturity appropriate for a rice-based cropping pattern. It assumes that a longer grain filling period would result in a larger number or weight of grains. Early heading and maturity and longer grain filling are desirable traits in South Asia, where timely seeding of wheat depends on optimum harvest time of the preceding rice crop (Hobbs and Giri, 1997).

Variation in BY, STY and GY offer potential for improving these traits using the landraces included in this study. Biomass yield is the sum of GY and STY. While GY is of primary concern to most rice breeders, STY is also important to rice growers in developing countries where rice straw has value as cattle feed and other domestic uses (mattress, bed cushion, court-yard fence, thatch roof, etc.). Rice genotypes with high GY and high STY are preferred over those with high GY but low STY. Farmers may even trade off some GY to obtain high STY. Although grain colour may have little economic value, it might carry some preference – for example, black colour is the least preferred while white and red are more preferred (personal communications with farmers).

The high correlation between GY and STY means that they can be improved simultaneously. Similarly, GY can be improved through careful selection for HI in breeding populations. The positive correlation of STY with GY and BY implies that genotypes with acceptable levels of both GY and STY should be selectable in future crossing programmes involving landraces.

High correlations between growth periods (DH, DM and DGF) with GY, STY and BY suggest that it may be difficult to produce high-yielding rice genotypes with early maturity. At the same time, the lower insignificant correlation between TGW and GY and other characters suggests that grain quality needs to be handled as a special trait while selecting for high yield and fine grain.

High but different values for the two diversity indices H and D for the two groups of landraces suggest that all 13 traits differentiated the landraces but with different levels of discrimination. The higher H value for the lowland group signifies a greater genetic richness among the lowland-adapted landraces compared to the hill group. However, this conclusion should be approached with caution because there were fewer landraces in the hill than in the lowland group. The analyses present some evidence for the exchange of landraces over time among communities living in the transition zones between hills and lowlands in Nepal. There has been a continuous movement of people between the hills and the lowlands in the history of Nepal. The landraces studied were grouped by the lowland or hill district they were collected from, which does not necessarily represent their place of origin in Nepal. Allard (1965) suggested that sharp differences could occur within small distances in response to edaphic changes, while Bennett (1999) indicated that the variation among ecological niches may account for intra-population variations. Thus differences among landraces within each group were expected, since specific micro-environment niches do occur within a given ecosystem. The tight clustering of several landraces indicates their probable adaptation to a specific geographical niche, supporting the observation of Bajracharya *et al.* (2006) that, in a high-altitude (2200–3000 m asl) district of Nepal, the landrace population of rice showed a narrow genetic base.

Landraces with a shared same name sometimes grouped together, but in many cases they were separated from each other. Thus the traditional system of naming landraces is not consistent, a fact that needs to be taken into consideration when characterizing germplasm.

Overall, the landraces possessed extensive variation for yield components and phenological traits. The lowland- and hill-adapted landraces represent two broad gene pools and contain useful genetic diversity for important agronomic traits. Agronomic traits could be substantially improved by the use of these gene pools in selective breeding. This largely unexplored collection of Nepalese rice landraces should provide significant diversity for the improvement of national and international germplasm.

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