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Agro-morphological diversity of Nepalese naked barley landraces

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Abstract

Background: Naked barley is a traditional, climate-resilient and highly nutritious crop of the high mountains of Nepal. Knowledge of agro-morphological diversity among the naked barley genotypes is fundamental for their efficient utilization in plant breeding schemes and effective conservation programs. The primary objective of this study is phenotypic characterization and diversity assessment of naked barley landraces in Nepal for pre-breeding purposes.

Methodology: Data on quantitative and qualitative traits of 25 naked barley landraces from diversity blocks established across the four mountainous locations of Nepal (Humla, Jumla, Dolakha and Lamjung) were subjected to calculation of descriptive statistics and multivariate analysis (UPGMA cluster analysis/principal component analysis). Frequency distribution of various categories of qualitative traits and Shannon–Weaver diversity indices were computed.

Results: The UPGMA cluster analysis using both quantitative and qualitative traits individually categorized the 25 naked barley landraces in five clusters in each case with no distinct regional grouping patterns in such a way that the landraces from same or adjacent regions of origin and collection amassed in different clusters. NGRC04894, Lamjung Local, NGRC02306, NPGR1579, NGRC02327 and NPGR1579 exhibited morphoagronomical superiority and potentiality for utilization as genitors in crop improvement programs. Principal component analysis revealed the quantitative traits, viz. grain yield, plant height and earliness, and qualitative traits, viz. grain color, overall phenotypic performance, lemma awn/hood and lemma awn barbs, to be the principal discriminatory characteristics of the Nepalese naked barley landrace collection. The Shannon–Weaver diversity index (H') ranged from 0.32 to 0.99 with a mean value of 0.73, inferring tremendous diversity in the collection for the qualitative traits.

Conclusion: The marked diversity observed among the Nepalese naked barley landraces could be utilized in crop improvement for various traits. The information generated complements the robust breeding program of competitive, stable and climate-resilient varieties of end users' preferences in different mountainous agro-ecologies and also bolsters the employment of innovative and proven participatory plant breeding approach using diversity kits and informal research and development kits to expand and promote the varietal choice options for expeditious benefits to the farmers in the high mountains, considering that only one naked barley variety, viz. Solu Uwa, has been released in Nepal to date.

Keywords: Naked barley, Diversity, Agro-morphological traits, Nepal

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Background

Hordeum L. is a widely distributed genus of the tribe Triticeae with 45 species and subspecies consisting of mostly weedy annual or perennial grasses adapted to the temperate zones of both the northern and southern hemispheres. Barley (*Hordeum vulgare* L. subsp. *vulgare*) has historically been the prevalent cereal grown in marginal agricultural areas [40]. It is the world's fourth most important cereal crop after wheat, rice and maize. A salient botanical feature of domesticated barley is that most cultivars have covered caryopses in which the hull (outer lemma and inner palea) is firmly adherent to the pericarp epidermis at maturity; but few cultivars are of the free-threshing variant called naked (hullless) barley.

Naked barley/hullless barley (*Hordeum vulgare* var. *nudum* Hook. f.; Nepalese vernacular name: Uwa/Mudule Jau) differs from hulled barley in that the loose husk cover of caryopses is easily separable upon threshing [5]. The domestication of naked barley is believed to have occurred after the hulled type around 6500 BC [40]. The hullless grain trait is governed by the single recessive gene '*nud*' located on the long arm of chromosome 7H [14]. A monophyletic origin of naked barley has been suggested [41] as a single mutation event either from wild barley (*H. vulgare* subsp. *spontaneum*) or from domesticated hulled barley. It is mainly cultivated in the higher Himalayan ranges of East Asia (India, Nepal, Bhutan and Tibet), China, Korea and Japan [42, 43] and also at low frequency in Ethiopia [44]. Its cultivation has been practiced for ages in the high mountains of Nepal due to its adaptability and its traditional and cultural importance. The barley landraces from the Himalayas, particularly from the highlands of Nepal, share a significant part of world barley germplasm resources [32]. As naked barley is extensively grown in the Himalayas of Nepal from the east to the west (about 800 km), along the Himalayas, it is frequently represented in Himalayan barley germplasm collections [23].

The importance of barley as a food crop increases with ascending altitude toward the north, where other cereals cannot be cultivated successfully due to poor edaphic factors and extreme environmental stress, particularly drought [21]. Naked barley is a traditional, culturally important, climate-resilient and highly nutritious winter cereal crop of the high mountainous region of Nepal. The unique geographical distribution pattern of naked barley in the higher altitudes (>2500 masl) implies its tremendous food value and wide utility in the high mountains. Furthermore, it does not require additional processing, contrary to hulled barley which has tightly adhered grain husks. It is rich in soluble β -glucans content, which acts as an inhibitor of cholesterol synthesis and thus is salubrious for heart patients [4, 5, 9]. Its integration in

daily diet can help to reduce blood glucose and cholesterol [20]. Gladiators also popular as *hordearii* (the eaters of barley) were known for their strength and stamina [1, 15]. In the highlands, its flour is mostly consumed as *Satu* (a flour prepared from roasted barley grains which can eaten as it is or can be used in several cuisines), flat breads, dumplings, *Timmok* and *Thukpa* (Tibetan noodle soup) and also mixed with flour of other crops like wheat, buckwheat and finger millet. Additionally, a special traditional fermented drink *Chhyang* and distilled liquor are prepared from the grains for different cultural and religious occasions by Lama, Karmarong and Sherpa tribes residing in mountainous region of Nepal [11, 22, 24, 25].

Due to its hardy nature, ability to grow well in low-input conditions and potential for use in climate change adaptation, naked barley renders itself as one of the future- and climate-smart crops in the wake of bulging food insecurity and climate change in the fragile mountainous region of Nepal. In Nepal, around 3.7 million people are estimated to be food-insecure [34]. Further, the food insecurity is critically alarming in far and mid-western mountains of Nepal [8] and likely to be aggravated more in days to come due to rapidly changing climate. In this context, the wider cultivation of this highly climate-resilient and nutritious crop could play an important role in climate change adaptation and ensuring food security in mountainous regions of Nepal. To date, only one naked barley variety, viz. Solu Uwa, has been released in Nepal with yield potential of 1.9 Mt/ha [18]. The inadequacy of promising and adaptive naked barley cultivars in national agricultural system has left mountainous farmers with nominal varietal selection options compelling them to shift to comparatively high-yielding food crops such as wheat and hulled barley, effecting a sharp decline in the cultivation trend of naked barley. Despite its tremendous potential and prospects, the crop has not been able to engross satisfactory attention regarding research and development in Nepal and remains underutilized and neglected.

The existence of the marked diversity in morphology and agronomic traits between and within the landrace population and the genetic uniqueness of Nepalese naked barley has been reported by various authors [3, 23, 29], which signifies abounding room for robust breeding and crop improvement program. Pandey et al. [23] reported a high level of genetic diversity and complex population structure in Himalayan hullless barley landraces. Sharma et al. [29] found a vast variation in morphology between and within landrace populations. Besides this, differences in agronomic performance and disease resistance were detected [3]. Xu et al. [38] collected 562 hullless barley accessions with worldwide geographic origins and evaluated their genetic variability and relatedness based on

93 SSR markers. However, precise and detailed information on the pattern and structure of the phenotypic and agro-morphological diversity of Nepalese naked barley landraces is still lacking and paltry information is available for pre-breeding purpose. Advances in molecular techniques have opened doors to genetic screening of germplasm collections. Yet, accurate phenotyping is of paramount importance for identifying useful traits [19], visualizing the pattern of variation in agro-morphological traits, realizing the relative significance of the germplasm in breeding programs and facilitating effective management, conservation and exploitation of the gene pool. Hence, the present study, which is a part of sourcing local crop diversity to match farmers' needs through rapid diversity assessment, on-farm evaluation and dissemination of choice varieties under a GEF/UNEP funded project on local crops in Nepal (www.himalayancrops.org), was undertaken to generate explicit estimates of the agro-morphological and phenotypic diversity of Nepalese naked barley landraces for pre-breeding purpose and efficient deployment of the functional diversity to broaden the genetic base of community richness using a participatory plant breeding approach. In addition, the local crop project aims for large-scale testing of the candidate plant materials in the wider areas of Nepal using diversity kits for deployment of new diversity in order to expand the varietal choice options of the farmer and enhance agricultural system resilience, appraisal of the agronomic performance via crowdsourcing feedback [33] or a quick sample household survey as those employed by Joshi et al. [13] and Witcombe et al. [35], and use of evolutionary plant breeding [26] or simple grassroots breeding [31] of the crop for rapid development of a competitive and stable variety to provide better varietal choices than the existing one in a particular agro-ecology.

Methods

Plant materials, site description and field experiment

The materials used in this study consisted of a total of 25 naked barley landraces collected from different agro-ecological zones of Nepal (Table 1). Of all landraces, 19 were provided by National Agricultural Genetic Resource Centre (NAGRC), 5 were collected from farmer's field of different locations, and 1 (Solu Uwa) was collected from Hill Crop Research Program (HCRP). On-farm diversity block trials were laid out in four mountainous locations, viz. Lamjung, Dolakha, Humla and Jumla in 2016. Diversity block is an experimental block of farmers' varieties managed by a local institution for research and development purposes [39]. The block is not only used for measuring and analyzing agro-morphological characteristics, but also used to validate farmers' descriptors by inviting farmers to watch the diversity block in the field and

determine whether farmers are consistent in naming and describing varieties. The four experimental locations are designated in Fig. 1. The geo-climatic description of the experimental locations is depicted in Table 2. Each landrace was continuously sown in 4 rows of 3 m length with the plot size of 3 m² with distance of 25 cm between row to row. All agronomic and management practices were followed as per the farmer's practice. Well-rotten farm yard manure @ 6–8 t ha⁻¹ was applied in the field during land preparation. No chemical fertilizer and pesticides were used. Two hand weeding were performed 30 and 60 days after sowing. The lack of seeds of various landraces brought about asymmetrical distribution/inclusion of the study materials in the experimental locations such that 19 landraces were tested in Lamjung, 18 in Humla, 20 in Dolakha and 22 in Jumla (Table 1).

Data collection and data analysis

Data on 5 quantitative traits such as days to emergence, days to heading, days to maturity, plant height (cm), grain yield (gplot⁻¹) and 10 qualitative characteristics such as growth habitat, stem pigmentation, tillering capacity, spike density, lemma awn or hoods, lemma awn barbs, glume color, grain color, grain size and overall phenotypic acceptance were recorded as per the descriptors of barley [12]. Grain yield was recorded after sun drying in gplot⁻¹ and later extrapolated to kgha⁻¹. A modified scale (3=good, 6=intermediate and 9=poor) for tillering capacity and overall phenotypic acceptance was employed. For analysis of quantitative data, mean values were used for the common landraces tested across the experimental locations while data of those landraces which were assessed in only one of the experimental locations were used as such. The qualitative data of 22 landraces tested in Jumla, 2 landraces (Rato Humla and Jungu Local) tested in Dolakha and one landrace (Lamjung Local tested) in Lamjung were further analyzed. The descriptive statistics for quantitative traits of the clusters and individual landraces were calculated. Multivariate analyses based on 5 quantitative and 10 qualitative data such as Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering based on similarity distance and principal component analysis (PCA) were performed in order to assess the degree of divergence and relatedness among the landraces and estimate the relative importance and contribution of traits to the overall variation using Minitab V17. Shannon–Weaver diversity indices [28] were calculated in order to estimate the phenotypic diversity for each qualitative trait with Microsoft Excel using the formula: Standard $H' = [\sum(n/N) \cdot \log_2(n/N) \cdot (-1)] / \log_2 k$, where H' is the standardized Shannon–Weaver diversity index, k is the number of phenotypic classes for a character, n is the

Table 1 Details of accessions of naked barley used in the study

Accession	Trial location	Local name	Origin		Altitude (masl)	Source
			District	Place name		
Acc6327	Hanku	Jau	Mustang	Dhumba	2800	NAGRC ^a
Co4710	All	Jau	Mustang	Marpha	2695	NAGRC
Co4712	All	Uwa	Mustang	Jharkot	2690	NAGRC
Co4713	All	Karu (gho)	Mustang	Khinga	3000	NAGRC
Co5351	All	Taksar	Okhaldhunga	Bigutar 2	1800	NAGRC
Co5503	All	Uwa	Dhading	Jharlang	1450	NAGRC
Co5565	All	Local uwa	Ramechhap	Gumdel 9	2500	NAGRC
Co5638	All	Uwa	Lamjung	Taghring 1	1700	NAGRC
Co5639	All	Local uwa	Lamjung	Ghanpokhara	1600	NAGRC
NGRC02306	All	Uwa	Mustang	Chosser	3800	NAGRC
NGRC02327	All	Uwa	Myagdi	Sukebagar	1280	NAGRC
NGRC04003	All	Jau	Mustang	Dhumba	2800	NAGRC
NGRC04894	All	Jau	Humla	Dadha phaya	3000	NAGRC
NGRC04902	All	Local uwa	Manang	Pisang-1	3000	NAGRC
NGRC04903	All	Jhuse	Mugu	Karki wada	1900	NAGRC
NPGR1579	All	Uwa	Mugu	Skypa gaun	3948	NAGRC
NPGR1604	All	Karu	Sindhupalchok	Ghanjwal	2450	NAGRC
NPGR1621	All	Uwa	Mustang	Chosser	3800	NAGRC
NPGR1626	All	Uwa	Mustang	Chaile	3000	NAGRC
Rato Humla	Jungu	Uwa	Humla	Chippra	2200	Farmer's field
Rato Kalo	Hanku	Uwa	Jumla	Hanku	1900	Farmer's field
Hanku Local	Hanku	Uwa	Jumla	Hanku	1900	Farmer's field
Jugu Local	Jungu	Uwa	Dolakha	Jungu	1800	Farmer's field
Lamjung Local	Ghanpokhara	Karu	Lamjung	Ghanpokhara	1650	Farmer's field
Solu uwa	Hanku and Chhipra	Solu uwa	Solukhumbu			HCRP ^b

^a National Agricultural Genetic Resource Centre (1350 masl), Khumaltar, Nepal

^b Hill Crop Research Program (1740 masl), Kabre, Dolakha, Nepal

frequency of a phenotypic class of that character and N is the total number of observations for that character.

Results and discussion

Pattern and structure of polymorphism for quantitative traits

Multivariate analyses, viz. UPGMA clustering and principal component analysis, were performed on a set of 25 Nepalese naked barley landraces by 5 quantitative traits, viz. days to emergence, days to heading, days to maturity, plant height and grain yield. The naked barley landraces were clustered into 5 distinct groups based on 5 quantitative traits (Fig. 2). The descriptive statistics of the distinct clusters are presented in Table 3. The number of landraces belonging to distinct clusters varied from 1 in Clusters IV and V each to 15 in Cluster I. Cluster I was the largest cluster comprising of 15 landraces (60%) followed by Cluster II with 6 landraces (24%). The landraces embraced in Cluster I depicted higher mean values for traits such as grain yield, plant height and moderate

mean values for other agronomic traits. Similarly, the members of Cluster II portrayed relatively lower mean values for grain yield and plant height while moderate mean values for other agronomic traits. Clusters III and IV consisted of 1 landrace each while Cluster V contained 2 landraces. Of all, Cluster IV consisting of only landrace, Lamjung Local revealed agronomic superiority entailing relatively higher grain yield (3610 kg ha⁻¹) and lower values for phenological traits. Lamjung Local transcended all other landraces in Lamjung conditions for having preeminence of yield and earliness (Table 4). Earliness is one of the crucial traits that is highly valued by farmers dwelling in dry and rain-fed conditions in the mountains that is useful to escape drought, hailstone damage and terminal heat stress. In addition to this, the cultivation of early cultivars of naked barley ensures timely planting of rice crop and having rice intact in the cropping pattern. Women prefer early cultivars as it ensures that the food is available during lean period. The selection of the breeding material from cluster I holds more reliable and

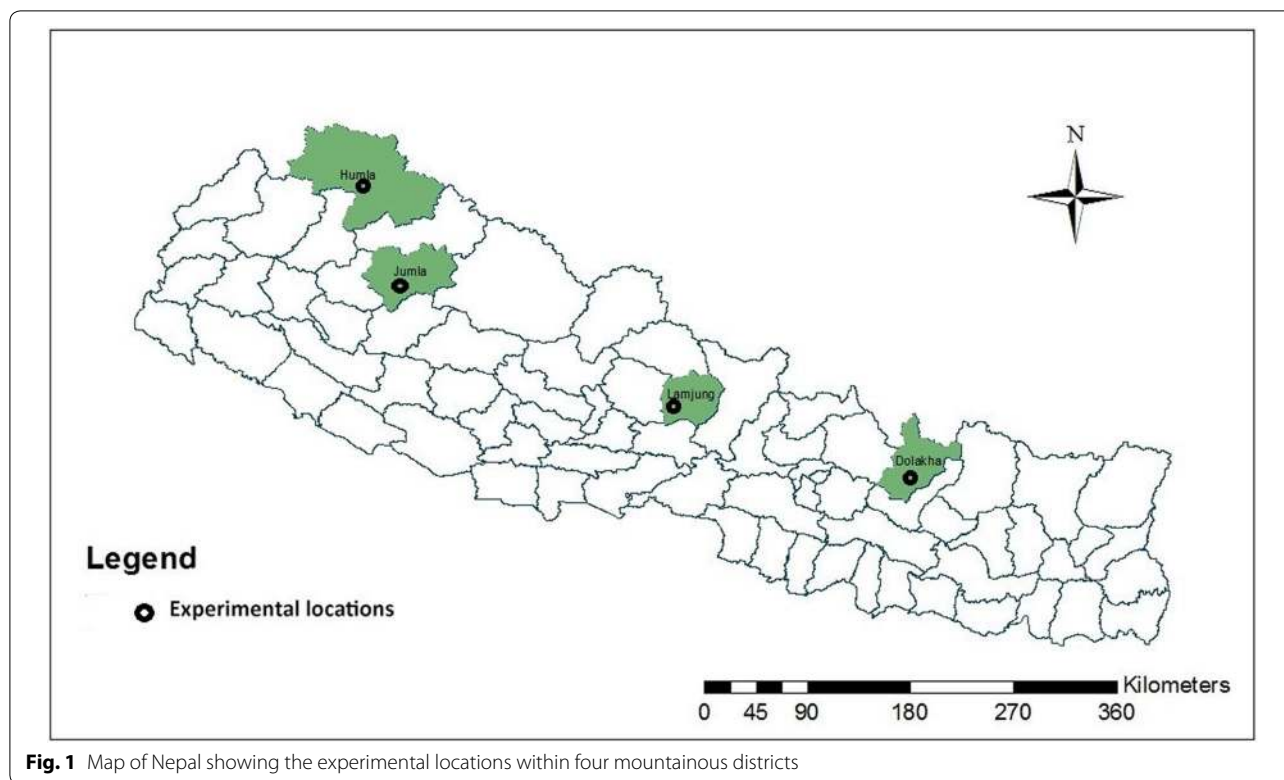


Fig. 1 Map of Nepal showing the experimental locations within four mountainous districts

Table 2 Geo-climatic description of the experimental locations

Site	Temperature (°C)	Average annual rainfall (mm)	Climate range	GPS	Altitude (masl)
Ghanpokhara, Lamjung	15–27	2944	Warm to cool temperate	N28°17.272'E84°19.249'	1621
Jungu, Dolakha	4–22	2000	Warm to cool temperate	N27°41.55'E86°09.62'	1792
Chhipra, Humla	0–20	50	Cool temperate to Alpine	N°59.107'E81°50.011'	2988
Hanku, Jumla	2–22	728.9	Cool temperate to Alpine	N29°14.329'E082°06.644'	2375

rational provided that the mean values of the quantitative traits for the cluster member across the multi-locations were used in the study. While considering the multi-environment trial data, the mean overall performance of landraces in Cluster I was appreciably better than the others. The UPGMA clustering of the landraces revealed ample variation for the agronomic traits and facilitated the selection of preferable landraces for pre-breeding and further evaluation. Several workers have deployed cluster analysis to depict the heterogeneity and similarity among the accessions and interrelationship among characters [7, 16, 36]. The analysis also revealed that the landrace aggregation was independent of the geographic source of collection, which is similar to the findings of Zakova and Benkova [37], Abebe et al. [1] and Mekonnon et al. [16]. Characterization of accessions and clustering of them on the basis of their morphological and genetic similarity

aids in identification and selection of the best parents for hybridization [30]. Hence, grouping of landraces using multivariate analysis such as UPGMA clustering would be valuable for the breeders in such a way that the most promising landraces in the population may be selected from different clusters for crop improvement.

The visualization of location-specific agronomic performance of these naked barley landraces facilitated the identification and selection of promising landraces/genitors for breeding programs, and further evaluation studies directed at validation and providing a wider varietal selection option for farming, in particular agro-ecology. The location-wise estimate of the major quantitative traits of the tested landraces that are highly valued by farmers for selection and adoption is presented in Table 4. Of all the test locations, Lamjung received the highest rainfall uniformly

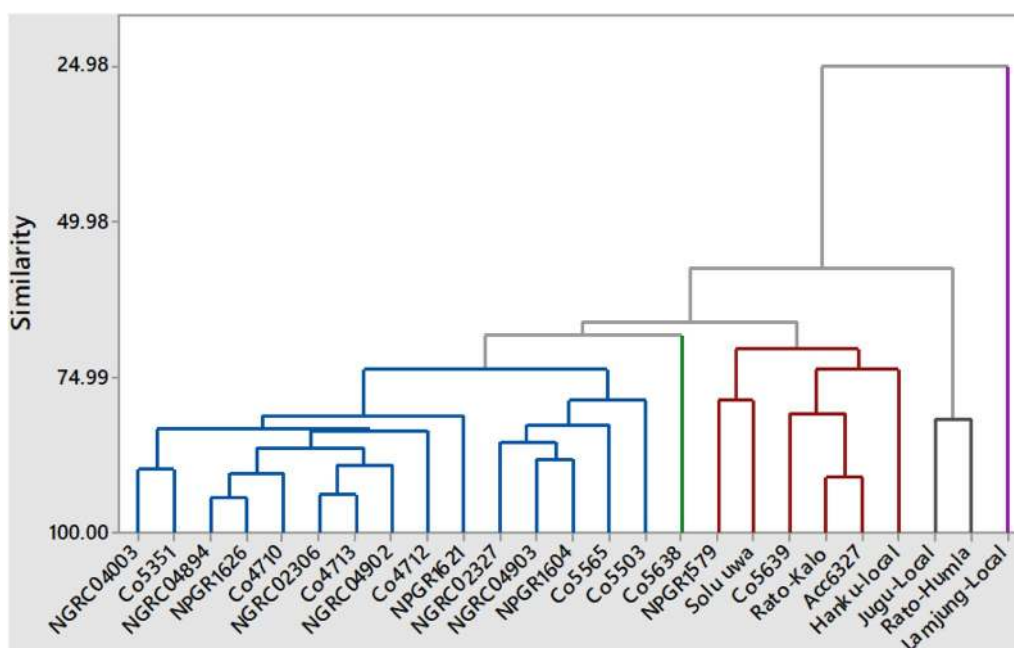


Fig. 2 UPGMA clustering based on quantitative traits using average linkage and Euclidean distance

Table 3 Descriptive statistics of quantitative traits within clusters of naked barley accessions

Clusters	Accessions	Mean ± S.D.				
		Days to emergence	Days to 50% heading	Days to 80% maturity	Plant height (cm)	Grain yield (kg/ha)
I	Co4710, Co4712, Co4713, Co5351, Co5503, Co5565, NGRC02306, NGRC02327, NGRC04003, NGRC04894, NGRC04902, NGRC04903, NPGR1604, NPGR1621 and NPGR1626 {15}	11.18 ± 0.27 (13–10)	109.68 ± 1.91 (119.25–94.75)	150.58 ± 1.36 (156.5–139)	86.20 ± 1.82 (100.8–78.52)	1816.30 ± 67.30 (2150–1347.1)
II	Acc6327, Hanku Local, Co5639, NPGR1579, Rato Kalo, Solu uwa{6}	14.58 ± 0.82 (17–11.5)	115.95 ± 3.15 (123.75–102)	151.70 ± 3.20 (166–147)	71.00 ± 1.02 (74.61–68.77)	1071.81 ± 138.05 (1633.33–600)
III	Co5638 {1}	17.5	110.75	147.25	95.26	1727.08
IV	Lamjung Local {1}	7	92	134	130	3610
V	Jugu Local, Rato Humla {2}	7	102.5 ± 5.55 (108–97)	170.5 ± 2.5 (173–168)	81.70 ± 1.30 (83.0–80.4)	1546.67 ± 166.66 (1713.33–1380)

Figures in parenthesis represent the range of values of traits for each cluster

Figures in italics in the curly bracket represent the number of members within each cluster

distributed year round and had fairly conducive temperature range during the cropping season as compared to the other sites (Table 2). Due to relatively favorable climatic conditions and better soil heath management

practices of farmers in Lamjung such as intercropping with legumes and improved farm yard manure management and application practices, grain yield data of the test landraces might have been recorded significantly

Table 4 Mean agronomic performance of naked barley accession in different locations of Nepal

Accessions	Dolakha			Lamjung			Jumla			Humla		
	DM ^a	PH ^b	YT ^c	DM	PH	YT	DM	PH	YT	DM	PH	YT
NGRC04003	142	108	1790	144	68	667	147	72	1000	175	77	313
NGRC02327	146	97	1370	133	91	1717	138	80	800	157	76	132
NGRC04894	151	91	1993	144	78	2493	145	73	1567	182	72	316
NGRC02306	151	87	1313	144	81	2990	147	74	1700	174	75	763
NGRC04903	149	98	1543	134	107	3383	140	71	900	170	79	555
NGRC04902	149	97	1517	142	96	3067	147	77	867	179	80	603
NPGR1604	145	89	1033	134	103	3043	140	76	900	172	77	557
NPGR1626	151	82	1173	146	89	2990	147	71	1600	180	83	758
NPGR1579	144	72	267	147	67	1020	147	58	200	177	78	2417
Co5351	146	86	1020	134	96	2567	141	73	1033	166	69	550
Co5638	142	107	470	138	108	3613	141	72	1100	168	94	1255
Co5639	142	74	533	135	87	2627	147	56	767	165	59	277
Co5565	139	107	790	134	131	3743	147	81	567	184	84	628
Co5503	139	105	1118	133	122	2227	138	82	1067	146	82	443
Co4712	139	95	1123	145	94	3973	147	64	1567	150	72	627
Co4713	137	79	1213	145	97	3467	147	74	1400	183	79	507
Co4710	147	86	1540	145	98	3707	147	73	1367	187	81	227
NPGR1621	147	98	860	146	116	3590	147	86	967	184	88	273
Solu uwa	–	–	–	–	–	–	147	72	1633	185	78	412
Lmjung Local	–	–	–	134	130	3610	–	–	–	–	–	–
Jugu Local	168	80	1713	–	–	–	–	–	–	–	–	–
Rato Humla	173	83	690	–	–	–	–	–	–	–	–	–
Rato Kalo	–	–	–	–	–	–	147	69	600	–	–	–
Acc6327	–	–	–	–	–	–	147	73	933	–	–	–
Hanku Local	–	–	–	–	–	–	140	83	1633	–	–	–

^a Days to maturity^b Plant height (cm)^c Yield (kg/ha)

higher in contrast to other experimental locations. NGRC04894 (1933.33 kg/ha⁻¹), Lamjung Local (3610.00 kg/ha⁻¹), NGRC02306 (1700.00 kg/ha⁻¹) and NPGR1579 (2416.67 kg/ha⁻¹) were found to be superior yielders in Dolakha, Lamjung, Jumla and Humla, respectively. Likewise, NGRC02327 (133 and 138 days) demonstrated considerable earliness in Lamjung and Jumla conditions. NPGR1579 revealed remarkably lower value for plant height (72, 67 and 58) in the test locations, viz. Dolakha, Lamjung and Jumla. These landraces with desirable trait value could be utilized for further validation and breeding programs.

The PCA was performed to estimate the relative importance and contribution of each quantitative trait to the total variance and illustrate the agronomic diversity among the 25 landraces. Table 5 depicts the principal component and percentage of contribution of each component to the total variation in the naked barley landraces. The first two principal components (PCs)

Table 5 Principal component analysis for quantitative traits of naked barley accessions

Quantitative trait	PC1	PC2	PC3
Eigen value	2.783	1.2231	0.5295
Proportion of variance	0.557	0.245	0.106
Days to emergence	0.357	0.658	-0.151
Days to 50% heading	0.484	-0.014	-0.755
Days to 80% maturity	0.287	-0.751	-0.128
Plant height (cm)	-0.536	0.059	-0.333
Grain yield (kg/ha)	-0.522	-0.023	-0.529

with Eigen value ≥ 1 accounted for 79.5% of the entire variability. The first PC explained 55.7% of the variance. Plant height, grain yield and days to heading contributed profoundly to the first PC. The second PC explained for 24.5% of the variance for which days to maturity and

emergence contributed heavily. The results of PCA suggested that traits, viz. grain yield, plant height and earliness, were the principal discriminatory characteristics of the Nepalese naked barley landraces. The findings are in concordance with the farmers’ perception and criteria for selection of a cultivar in high mountains. Similar inferences, i.e., recognition of patterns in variability in barley traits via PCA, were obtained by several authors [2, 6, 36].

Pattern and structure of polymorphism in qualitative traits

Similar multivariate analyses as employed in the former section, viz. UPGMA and PCA, were performed to uncover major qualitative traits contributing to the diversity of the landrace. The qualitative traits taken into account for analyses were growth habitat, stem pigmentation, tillering capacity, spike density, lemma awn or hoods, lemma awn barbs, glume color, grain color, grain size and overall phenotypic acceptance. The UPGMA cluster analysis based on similarity distance

generated 5 distinct clusters with similar patterns of qualitative traits in each (Fig. 3 and Table 6). The number of landraces belonging to each cluster varied from 1 in Cluster II and IV each to 16 in Cluster I. Cluster I (64%), the largest of all, containing 16 landraces predominantly delineated erect growth habit, green and purple stem pigmentation, high tillering capacity and intermediate grain size with yellow glume color. Cluster III consisting of 5 landraces was principally characterized with purple stem pigmentation, low tillering and black/purple pericarp. Cluster V comprising 2 landraces demonstrated purple stem pigmentation, low tillering and small grain size. Similarly, Clusters II and IV comprising 1 landrace each were distinguished by revealing a unique set of quantitative traits as illustrated in Table 6. Although the cluster analysis grouped the naked barley landraces with greater phenotypic similarity, the cluster did not necessarily contain all the landraces from the same or adjacent niche/source of collection. This finding is in harmony with Dejene

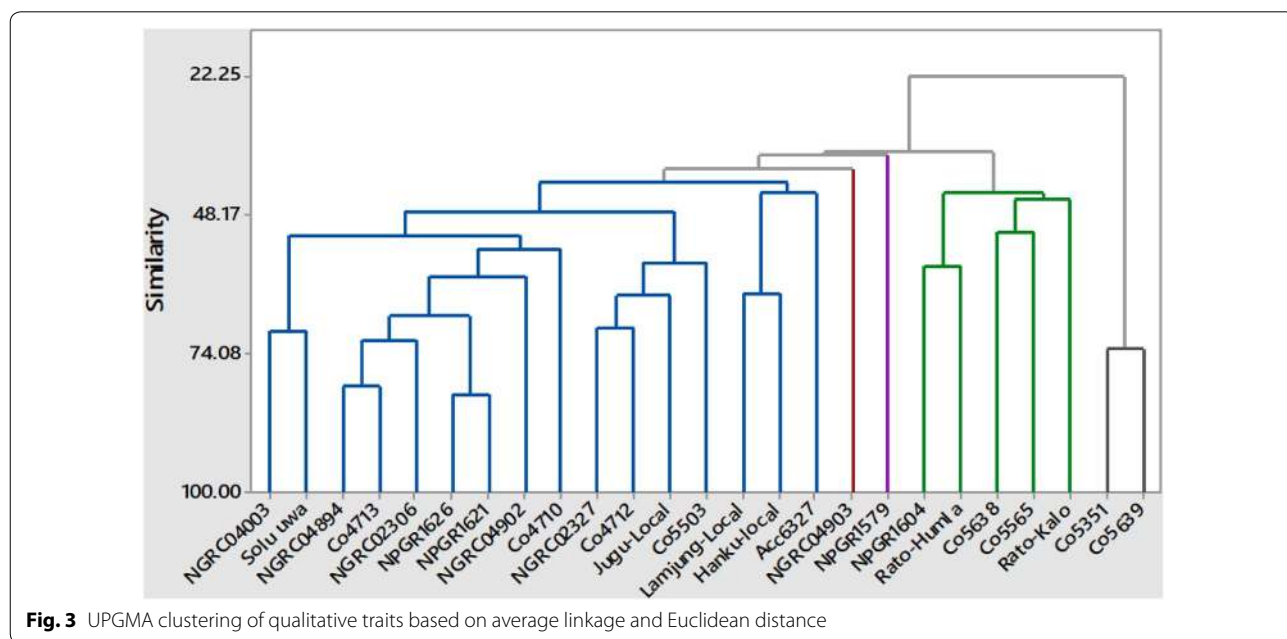


Fig. 3 UPGMA clustering of qualitative traits based on average linkage and Euclidean distance

Table 6 Characterization of clusters of naked barley accessions based on qualitative traits

Cluster	Accession	Major qualitative traits
I	Acc6327, Co4710, Co4712, Co4713, Co5503, Hanku Local, Jugu Local, Lamjung Local, NGR C02306, NGR C02327, NGR C04003, NGR C04894, NGR C04902, NPGR1621, NPGR1626, Solu uwa (16)	Erect type of growth habit, stem pigmentation green and purple, high tillering capacity, intermediate grain size with yellow glume color
II	NGR C04903 (1)	Large grain size, tan/red grain pericarp color
III	Co5638, Co5565, NPGR1604, Rato Humla, Rato Kalo (5)	Purple stem pigmentation, low tillering, black/purple grain pericarp
IV	NPGR1579 (1)	Prostrate growth habit, high spike density, tan-red color, large grain
V	Co5351, Co5639 (2)	Purple stem pigmentation, low tillering capacity, small grain size

et al. [1] and Mekonnen et al. [16], who reported that the clustering of accessions based on the agronomic characters revealed no distinct regional grouping patterns in which accessions from same or adjacent regions appeared in different clusters. The discrimination of landraces into discrete clusters concluded preponderance of polymorphism among the germplasm of Nepalese naked barley landraces for qualitative traits. Additionally, a good fit between the graphical representation of distances and their similarity matrices was noticed in the dendrogram (Fig. 3). Knowledge on degree of genetic relatedness among the genotypes provides robust information to address breeding programs and genetic resource management [27].

The PCA based on the qualitative traits revealed that the first four PCs with Eigen value ≥ 1 accounted for 69.80% of the total variance (Table 7). For the first PC accounting for 27.5% of the total variance, the traits such as grain color, overall phenotypic acceptance, lemma awn/hood and stem pigmentation posed the largest contribution in the positive direction while lemma awn barbs and growth habit loaded heavily in the negative direction. The second PC accounting for 18.3% of the total variance was mainly influenced by growth habit, tillering capacity and lemma awn barbs while the traits loading heavily on the third PC (13.7%) were spike density and stem pigmentation. Similarly, the fourth PC accounted for 10.30% of the total variance for which stem pigmentation and growth habit posed the largest load. The comparison between the clusters and groups developed from PCA allowed us to deduce that the set of qualitative traits contributing substantially to divergence in naked barley landraces

included grain color, overall phenotypic acceptance, lemma awn/hood and lemma awn barbs.

Estimate of phenotypic diversity

Substantial polymorphism was revealed across all the qualitative traits with varying degrees of contribution to the phenotypic diversity. Descriptor states and their frequency as well as Shannon–Weaver diversity indices for each qualitative trait are depicted in Table 8. The diversity index (H') ranged from 0.32 to 0.99 with a mean value of 0.73, inferring tremendous diversity in the collections for the qualitative traits. The estimate of Shannon–Weaver diversity indices (H') considers both richness and evenness of the phenotypic classes of the traits. High H' was inferred for most of the traits such as grain size ($H'=0.99$), phenotypic acceptance ($H'=0.99$), spike density ($H'=0.94$), stem pigmentation ($H'=0.92$), growth habit ($H'=0.89$) and tillering capacity ($H'=0.83$) while moderate values of H' were revealed for lemma awn barbs ($H'=0.62$) and glume color ($H=0.53$). However, relatively lower values of H' were found for pericarp color ($H'=0.37$) and lemma awn/hood ($H'=0.32$). The notable phenotypic diversity in Nepalese naked barley landraces may be attributed to high agro-ecological heterogeneity, propitious for the cultivation of crops in Nepal. In addition to this, the findings showed that the structure of morphological variation in Nepalese naked barley landraces is significantly influenced by natural selection factors. Tremendous agro-morphological variation in Nepalese barley landraces has been reported by several authors [10, 17]. The existence of high phenotypic diversity in the Nepalese naked barley landrace increases the space for selection and pre-breeding purpose of the elite varieties with earnest emphasis to the farmers' preferences of the decisive morphological traits in the selection and adoption process. Morphological traits have been extensively taken into account by farmers *inter alia* to discriminate varieties regarding selection and adoption of a particular variety [17]. High mean value of Shannon–Weaver index ($H'=0.74$) for the qualitative traits confirmed huge genetic diversity in the collection and more or less balanced frequency classes for individual traits with implications for utilization in robust crop improvement and enhancement of genetic potential of naked barley.

Conclusion

The present study engendered clear insights on the pattern and structure of agro-morphological and phenotypic diversity in collection of 25 Nepalese naked barley landraces, which is very crucial for efficient pre-breeding, management and utilization in the crop improvement program and enhancement of genetic potential of

Table 7 Principal component analysis of naked barley accessions based on qualitative traits

Quantitative traits	PC1	PC2	PC3	PC4	PC5
Eigen value	2.74	1.82	1.37	1.03	0.81
Proportion of variance	0.27	0.18	0.13	0.10	0.08
Growth habit	-0.21	-0.49	0.05	-0.37	-0.11
Stem pigmentation	0.32	-0.12	0.31	-0.57	0.11
Tillering capacity	-0.30	0.44	-0.09	-0.27	0.02
Spike density	0.11	-0.06	-0.67	0.04	-0.53
Lemma awn/hoods	0.42	0.19	-0.09	-0.07	0.25
Lemma awn barbs	-0.38	-0.39	-0.09	-0.30	-0.03
Glume color	0.01	-0.48	0.20	0.54	0.02
Grain color	0.45	-0.23	-0.03	0.06	0.17
Grain size	-0.06	0.22	0.61	0.11	-0.57
Overall phenotypic acceptance	0.45	-0.06	0.02	-0.20	-0.51

Table 8 Descriptor states and Shannon–Weaver index of qualitative traits of naked barley accessions

SN	Qualitative traits	Shannon–Weaver index	Descriptor's states	Frequency	Proportion
1	Growth habit	0.89	Prostrate	3	12
			Intermediate	8	32
			Erect	14	56
2	Stem pigmentation	0.92	Green	13	52
			Purple (basal only)	5	20
			Purple (half or more)	7	28
3	Spike density	0.94	Lax	5	20
			Intermediate	12	48
			Dense	8	32
4	Lemma awn/hood	0.32	Awnless	0	0
			Awnleted	1	4
			Awned	21	84
			Sessile hoods	0	0
			Elevated hoods	3	12
5	Lemma awn barbs	0.62	Smooth	11	44
			Intermediate	9	36
			Rough	5	20
6	Glume color	0.53	White	3	12
			Yellow	18	72
			Brown	1	4
			Black	3	12
7	Grain(pericarp) color	0.37	White	2	8
			Tan/red	15	60
			Purple	1	4
			Black	2	8
			Other	5	20
8	Grain size	0.99	Small	9	36
			Intermediate	8	32
			large	8	32
9	Tillering capacity	0.83	Low	11	44
			Medium	2	8
			High	12	48
10	Phenotypic acceptance	0.90	Good	5	20
			Intermediate	14	56
			Poor	6	24

the crop. Our studies revealed the existence of tremendous agro-morphological diversity among the Nepalese naked barley landraces that could be utilized in effective crop improvement for various traits. Our studies substantiated that indirect selection of naked barley genitors based on various agro-morphological traits such as plant height and earliness, grain color, overall phenotypic performance, lemma awn/hood and lemma awn barbs could be worthwhile for crop improvement. The diverse landraces which were identified as superior in various agronomic traits in the present study

could be effectively utilized as genitors in hullless barley breeding. Furthermore, the information we generated complements the robust breeding program of high-yielding and climate-resilient varieties of end users' preferences and bolsters employment of innovative and proven participatory plant breeding approaches using diversity kits and IRD kits to expand and promote the varietal choice options for expeditious benefits and impact, fostering agro-biodiversity and food security in the high mountains.

Abbreviations

UPGMA: unweighted pair group method with arithmetic mean; PC: principal component; PCA: principal component analysis; masl: meter above sea level; NFC: Nepal Food Corporation; WFP: World Food Program; FAO: Food and Agriculture Organization; OGTR: Office of the Gene Technology Regulator; NARC: Nepal Agricultural Research Council; NAGRC: National Agricultural Genetic Resource Centre; HCRP: Hill Crop Research Program; t: tonne; ha: hectare; cm: centimeter; m: meter; IPGRI: International Plant Genetic Resource Institute; kg: kilogram; g: gram; IRD: international research and development; *H'*: Shannon–Weaver diversity index; Mt/ha: metric tonne per hectare; t ha⁻¹: tonne per hectare; g plot⁻¹: gram per plot; %: percent; kg ha⁻¹: kilogram per hectare; S.D.: standard deviation.

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Authors' contributions

RKY, SG, EP, RG, ARA and NP are the principal researchers who carried out the field experiments in multi-locations and collected the data. RKY performed the statistical analyses and helped SG in compilation of the data, interpretation of the results and drafting the manuscript. KHG and BKJ are the senior scientists who conceptualized and designed the whole study. RD is a plant breeder who helped RKY in statistical analyses and drawing inferences. EP, RG, ARA and NP also helped RKY and SG in refining the manuscript through their critical comment and suggestions. All authors read and approved the final manuscript.

Availability of data and materials

The data analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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