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RESEARCH ARTICLE

Nepalese foxtail millet [*Setaria italica* (L.) P. Beauv.] genetic diversity revealed by morphological markers

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Abstract Foxtail millet [*Setaria italica* (L.) P. Beauv.] is among the oldest cereal grains grown from time immemorial in the Himalayan regions of Nepal. However, Nepalese farmers do not have any improved variety officially released due to lack of substantial research in this crop. A total of 41 foxtail millet accessions were characterized using phenotypic or morphological markers at National Agriculture Genetic Resources Centre (Genebank), Khumaltar (1360 m a.s.l.), Lalitpur, Nepal during 2015 summer to enhance the utilization of foxtail millet genetic resources. Seven quantitative and nine qualitative traits were recorded using standard descriptors of foxtail millet to assess the intra-specific diversity. Significant diversity was observed among the accessions as revealed by Shannon–Weaver diversity indices (H′) for quantitative traits and qualitative traits. Six elite landraces in Cluster-4 and Cluster-5 (three from Lamjung district, and one each from Gorkha, Humla and Jumla districts) produced average grain yield of 3136 kg/ha at Khumaltar condition with earlier maturity (average of 89 days), taller height (average of 172 cm) and thicker panicles (average width of 27 mm). These landraces were selected for further evaluation in the farmers' field of mountain region due to their early maturity, higher yield potential, disease resistance and attractive panicles which could be considered as important genetic resources to develop climate resilient varieties to cope with the adverse effects of climate change.

Keywords Foxtail millet · Intra-specific diversity · Morphological markers · Nepal · Quantitative and qualitative traits

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Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv.], regarded as a native of China, is one of the world's oldest cultivated crops. It was originated in China, where it's domestication took place about 8000 YBP (Li and Wu 1996) as well as in south west Asia and central Europe (Vavilov 1926; Zohary et al. 2012) where it's cultivation recorded about 5000 YBP (Hammer et al. 1999). The Hindukush Himalayan region is rich in foxtail millet diversity (Scheibe 1943; Hammer and Khosbhakht 2007). The crop has moved from China to Northern India and Nepal during 5th to 2nd Millennium BC (Stevens et al. 2016), thus the Nepalese foxtail millet, mostly is *Setaria italica* subsp. *italica* race *maxima* (de Wet et al. 1979). It is the second most widely planted species among millets in the world and the most important millet in East Asia (Kumari et al. 2011; Ning et al. 2015; Sheikh and Singh 2013; Xiaomei et al. 2016; Zhang et al. 2014). It is known for its better tolerance to abiotic stresses compared to other cereal crops. It is also known as Italian millet, German millet or Hay millet but locally known as *Kaguno* in Nepal. Foxtail millet grain contains 12.3% protein, 4.3% fat, 60.9% carbohydrates, 14.0% dietary fibre and 3.3% minerals, with 31 g calcium, 290 mg phosphorus, 5 mg iron and vitamins than major staple crops rice and wheat (Saha et al. 2016; Saud 2010). Foxtail millet is the third important crop among group of millets with wide range of utility in Nepal. The cooked grain is used as *bhat* (cooked like rice), *dhindo* (porridge) and *kheer* (like rice pudding). Foxtail millet is valued by mountain farmers for its nutritional content and health promoting properties, ability to grow under low external input conditions and tolerance to extreme environmental stress, particularly drought. It is also recently appreciated because of medicinal benefits such as reducing blood glucose levels and cholesterol control in normal as well as diabetic patients. It is the crop of future in the context of changing climate with great potentiality to cope with food insecurity in remote areas of the country (Goron and Raizada 2015).

In Nepal, this crop is considered as traditional climate resilient and nutritionally dense crop but trends of cultivation and use are shrinking fast due to globalization, land use change, out migration, social values, change in food habit, depleting traditional knowledge, lack of research and policy support such as crop improvement and formal seed distribution system (Gurung et al. 2016; Parajuli et al. 2017; Palikhey et al. 2016; Sheikh and Singh 2013; Bisht et al. 2006). Precise data of area and production under foxtail millet is not known because the production statistics of this crop had often been grouped with other millets. Major foxtail millet growing districts in Nepal are Mugu, Kalikot, Humla, Jumla, Bajhang, Bajura, Dolpa, Lamjung, Gorkha, Ramechhap, Kavre, etc. where crop is grown sole as well as mixed with finger millet, proso millet, beans, amaranths, maize etc. Nepal is one of the centres of diversity of foxtail millet (Nakayama et al. 1999) and has high genetic diversity of this crop (Mo FSC 2002), however, very little research has been conducted and its status is still unexplored in Nepal thus called neglected and underutilized crop (Amgai et al. 2011). Plant genetic resources are conserved so that they can be used to improve crop production and in other ways. However, Hodgkin et al. (2003) asserted that use of ex situ conserved germplasm is inadequate globally and that genetic diversity maintained in genebanks is underutilized.

Poor utilization of local genetic resources conserved in genebank for foxtail millet improvement and development program is evident in Nepal due to (1) absence of public and private sector breeding priority, and (2) unavailability of characterization and evaluation information for wider use. GEF-supported Local Crop Project (www.himalayancrops.org), executed jointly by Nepal Agricultural Research Council, Department of Agriculture, LI-BIRD and Bioversity International, therefore aims to fill this gap and re-introduce genebank materials of foxtail millet landraces in traditional farming system for improving farmers access to diversity and increasing on-farm diversity. The objective of this study is to characterize diverse farmer's varieties of foxtail millet using agro-morphological data and use information to deploy useful diversity by participatory methods, and also for the efficient use of these accessions in future crop improvement programme.

Materials and methods

A total of 41 foxtail millet accessions were collected from six districts of Nepal (Fig. 1). Among them, 28 accessions were from Humla through diversity fair, six accessions were from Jumla, four accessions were from Lamjung and one accession each from Bajhang, Gorkha and Kavre districts. The detailed sources of accessions are presented in Table 1. These accessions were characterized ex situ at Genebank, Khumaltar (N27.4°, E085.2°, 1360 m a.s.l.), Nepal during summer 2015. Accessions were planted on 17th May, 2015 with an individual plot size of 6 m^2 following row to row spacing of 25 cm and plant to plant spacing of 10 cm. Fertilizers were applied at the rate of 20:30:20 kg/ha N:P_2O_5:K_2O as basal dose during

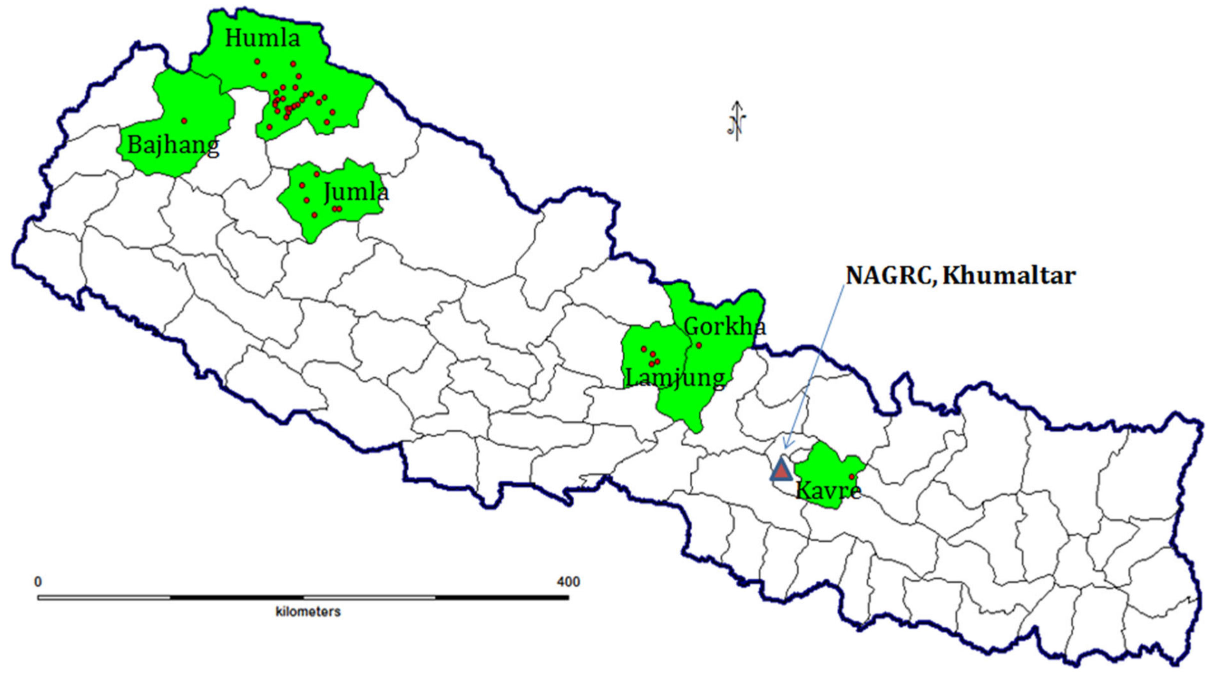

Fig. 1 Collection map of experimental materials and characterization location

land preparation and 20 kg/ha N was top-dressed 25 days after seeding. Different quantitative traits like days to 50% heading, days to 80% maturity, plant height (cm), panicle length (cm), panicle width (mm), panicle exertion (cm) and grain yield (kg/ha) as well as qualitative traits like growth habit, flag leaf angle, sheath pubescence, leaf pubescence, grain colour, grain yield potential, leaf blast susceptibility, stem borer susceptibility and overall phenotypic acceptability were recorded as per descriptors for *Setaria italica* (L.) P. Beauv. and *S. pumila* (Poir.) Roem. et Schult. (IBPGR 1985). Observations on days to heading, maturity and grain yield were based on whole plot data whereas observations on rest of the traits were from 10 randomly selected plants.

Shannon–Weaver diversity indices (Shannon and Weaver 1949) were calculated for each trait with Microsoft Excel using the formula: $H' = [\sum(n/N)^{*}\{log2(n/N)^{*}(-1)\}]/log2k$; where, H' is the standardized Shannon–Weaver diversity index, k is the number of phenotypic classes for a character, n is the frequency of a phenotypic class of that character and N is the total number of observations for that character. For the quantitative traits, accessions were divided into 10 phenotypic classes as $< x - 2sd$, $x - 2sd$, $x - 1.5sd$, $x - sd$, $x - 0.5sd$, x, $x + 0.5sd$, $x + sd$, $x + 1.5sd$, $x + 2sd$ and $> x+2sd$ are as the margins of the classes, where x is average and sd is standard deviation. The descriptive statistics, clustering, Principal Component Analysis (PCA) and correlation analysis is done by using Minitab-14.

Results and discussion

Descriptive statistics and Shannon–Weaver diversity

Nepalese foxtail millet germplasm found diverse in shape, size and colour of panicles as well as grains which has been illustrated in Fig. 2.

Range and average of observations as well as Shannon–Weaver diversity indices for different quantitative traits has been presented in Table 2. Flowering of accessions ranged from 45 to 71 days with the mean of 60 days from seeding. This range was wider than the flowering range of Nepalese accessions (45–60 days) but much narrower than the global collections (32–135 days) in ICRISAT, India (545 m) reported by Reddy et al. (2006). According to that report, the earliest maturity accession was from Russia

Table 1 Origin and sources of experimental materials

Accession	Local name	Collection site	Altitude (m a.s.l.)	Distinguishing trait
C1896	Aule Kaguno	Chandannath, Jumla	2290	Panicle branching in tip
C2578	Local Kaguno	Mangaltar, Kavre	1058	Brown grains
C3474	Seto Kaguno	Sunkuda, Bajhang	1764	White grains
C4576	Rato Kaguno	Patmara, Jumla	2498	Golden grains
C4577	Local Kaguno	Narakot, Jumla	2375	Red grains
C4578	Rato Kaguno	Birat, Jumla	2505	Red grains
C4580	Local Kaguno	Guthichaur, Jumla	2779	Light red grains
C4581	Rato Kaguno	Patmara, Jumla	2495	Straw white grains
C5148	Kalo Kaguno	Kharpunath, Humla	2750	Black panicle, white seed
C5643	Local Kauno	Gilung, Lamjung	1550	White grains
C5644	Local Kauno	Baglungpani, Lamjung	1650	Straw white grains
C5645	Seto Kauno	Ghanpokhara, Lamjung	1720	White grains
C5647	Local Kauno	Taghring, Lamjung	1500	White grains
C5808	Seto Kauno	Simjung, Gorkha	1850	White grains
H21	Kalo Kaguno	Melchham, Humla	2300	Drooping panicle
H23	Kalo Kaguno	Melchham, Humla	2350	Black panicle, white seed
H76	Local Kaguno	Gothi, Humla	2300	Brown grains
H77	Kalo Kaguno	Gothi, Humla	2300	Black grains
H85	Rato Kaguno	Gothi, Humla	2300	Red grains
H149	Rato Kaguno	Raya, Humla	2650	White grains
H150	Kalo Kaguno	Raya, Humla	2600	Black grains
H163	Kalo Kaguno	Chhipra, Humla	2400	Black panicles
H164	Piyalo Kauni	Chhipra, Humla	2450	Yellow panicles
H213	Kalo Kaguno	Kharpunath, Humla	2800	Black grains
H250	Rato Kaguno	Lali, Humla	2600	Light red grains
H251	Seto Kaguno	Lali, Humla	2600	White grains
H252	Kalo Kaguno	Lali, Humla	2600	Black grains
H314	Rato Kaguno	Saya, Humla	2000	Brown red grains
H378	Local Kaguno	Darma, Humla	1980	Single drooping panicle
H379	Pahelo Kauni	Darma, Humla	2000	Spines in panicle
H380	Kalo Kaguno	Darma, Humla	1980	Short plant
H468	Kalo Kaguno	Dandafaya, Humla	2350	Black drooping panicles
H469	Seto Kaguno	Dandafaya, Humla	2350	White grains
H522	Seto Kaguno	Syanda, Humla	2400	White grains
H523	Rato Kaguno	Syanda, Humla	2401	Red grains
H524	Kalo Kaguno	Syanda, Humla	2402	Black grains
H606	Kalo Kaguno	Sarkideu, Humla	1950	Black grains and panicles
H631	Kaguni	Kharpunath, Humla	2800	Straw white grains
H642	Kalo Kaguno	Kharpunath, Humla	2800	Drooping panicle
H708	Pahelo Kauni	Simikot, Humla	2900	Yellow grains
H709	Kalo Kaguno	Simikot, Humla	2900	Dark brown grains

Fig. 2 Diversity in panicle and grain types of Nepalese foxtail millet

Table 2 Descriptive statistics and Shannon–Weaver diversity index (H′) of 8 quantitative traits

Traits	Minimum	Maximum	Mean ± SE	H′
Days to 50% heading	45	71	60 ± 1.1	0.835
Days to 80% maturity	81	113	98 ± 1.8	0.806
Plant height (cm)	108	232	159 ± 4.2	0.852
Panicle length (cm)	13	30	21.7 ± 0.6	0.843
Panicle width (mm)	10	33	18.2 ± 0.8	0.820
Panicle exertion (cm)	7	30	15.9 ± 0.8	0.899
Grain yield (kg/ha)	89	3483	1247 ± 161	0.689

and the longest maturity was from Sri Lanka. This is very important adaptive trait for mountain farmers in rainfed dryland conditions, and also to escape for extreme cold temperature. Women farmers prefer earliness because the food is available during lean period. Plant height ranged from 108 to 232 cm with an average of 159 cm which is quite taller than the Nepalese (70–150 cm) as well as global (20–215 cm) accessions (Reddy et al. 2006). Our Nepalese accessions showed narrower range of panicle exertion (7–30 cm) as compared to the range in global collection of 1–36 cm (Reddy et al. 2006). Panicle length ranged from 13 to 30 cm which is similar to the range in Nepalese collection (13–25 cm) but narrower than the range in Indian collection (2–39) as reported by Reddy et al. (2006). Panicle width in our result showed narrower range (10–33 mm) wider than the range in Nepalese (10–25 mm) and very much narrower than the global (5–120 mm) collection (Reddy et al. 2006). Grain yield in our study ranged from 89 to 3483 kg/ha with the average of 1247 kg/ha. This is the preliminary yield from small plot (6 m^2) non-replicated trial in high fertility condition of Khumaltar. Some accessions were severely damaged by leaf blast (*Pyricularia setariae* Nisikado.) and false smut (*Ustilago crameri* Korn.) diseases and gave very poor yield.

Shannon–Weaver diversity index (H') considers both richness and evenness of the phenotypic classes of the traits. H' for all quantitative traits is ranged from 0.689 to 0.899 (Table 2) that for qualitative traits ranged from 0.443 to 0.989 (Table 3), suggested that characterized 41 foxtail millet accessions were diverse for recorded traits.

Clustering observations

UPGMA clustering divided 41 accessions of foxtail millet into five clusters (Fig. 3). Two clusters, Cluster-1 and Cluster-2, comprised of 15 accessions in each cluster mainly from Humla (Table 4). Cluster-1 has accessions that were low to intermediate yielder, medium maturity and height. Accessions from Cluster-2 were very low yielder (89–500 kg/ha), shortest panicle width (11–19 mm) and mainly late in maturity

Table 3 Shannon–Weaver diversity index, descriptor states and frequency of 9 qualitative traits

Qualitative traits	H′	Descriptor states	Frequency	Proportion (%)
Flag leaf angle	0.634	1. Erect	5	12.2
		2. Intermediate	26	63.4
		3. Horizontal	10	24.4
		4. Descending	0	0.0
Growth habit	0.797	1. Decumbent	3	7.3
		2. Erect	15	36.6
		3. Erect geniculate	23	56.1
Leaf pubescence	0.593	1. Dense	26	63.4
		2. Medium	15	36.6
		3. Sparse	0	0.0
Sheath pubescence	0.839	1. Dense	4	9.8
		2. Medium	22	53.7
		3. Sparse	15	36.6
Grain color	0.692	1. White	7	17.1
		2. Straw	6	14.6
		3. Brown	7	17.1
		4. Dark brown	13	31.7
		5. Light red	7	17.1
Grain yield potential	0.989	1. Low	16	39.0
		2. Medium	11	26.8
		3. High	14	34.1
Leaf blast susceptibility	0.558	1. Highly resistant	5	12.2
		2. Resistant	31	75.6
		3. Susceptible	4	9.8
		4. Highly susceptible	1	2.4
Stem borer susceptibility	0.443	1. Highly resistant	34	82.9
		2. Resistant	9	22.0
		3. Susceptible	0	0.0
Overall phenotypic acceptance	0.928	1. Good	7	17.1
		2. Intermediate	19	46.3
		3. Poor	15	36.6

(81–113 days). Cluster-4 and Cluster-5 jointly comprised of 6 accessions (3 from Lamjung, 1 each from Gorkha, Humla and Jumla districts) were of higher grain yield (2767–3473 kg/ha), taller plants (158–194 cm), greater panicle width (19–33 mm) and early maturity (86–99 days). Accessions from these two clusters can be further evaluated on-farm for their validation.

Principal component analysis

Table 5 shows the principal component analysis with eigen vectors, eigen values and variances due to first five principal components. In the first principal component, grain yield, panicle width and plant height with negative loading of − 0.581, − 0.486 and – 0.435, respectively were the most important traits. Similarly in the second principal component, days to heading with negative loading of − 0.639 and panicle length with positive loading of 0.496 were the most important traits. Panicle exertion was the most important trait in the third principal component followed by days to maturity with positive loadings of 0.871 and 0.374, respectively. Eigen analysis of the correlation matrix revealed that five principal components explained 95% of the total variance among the

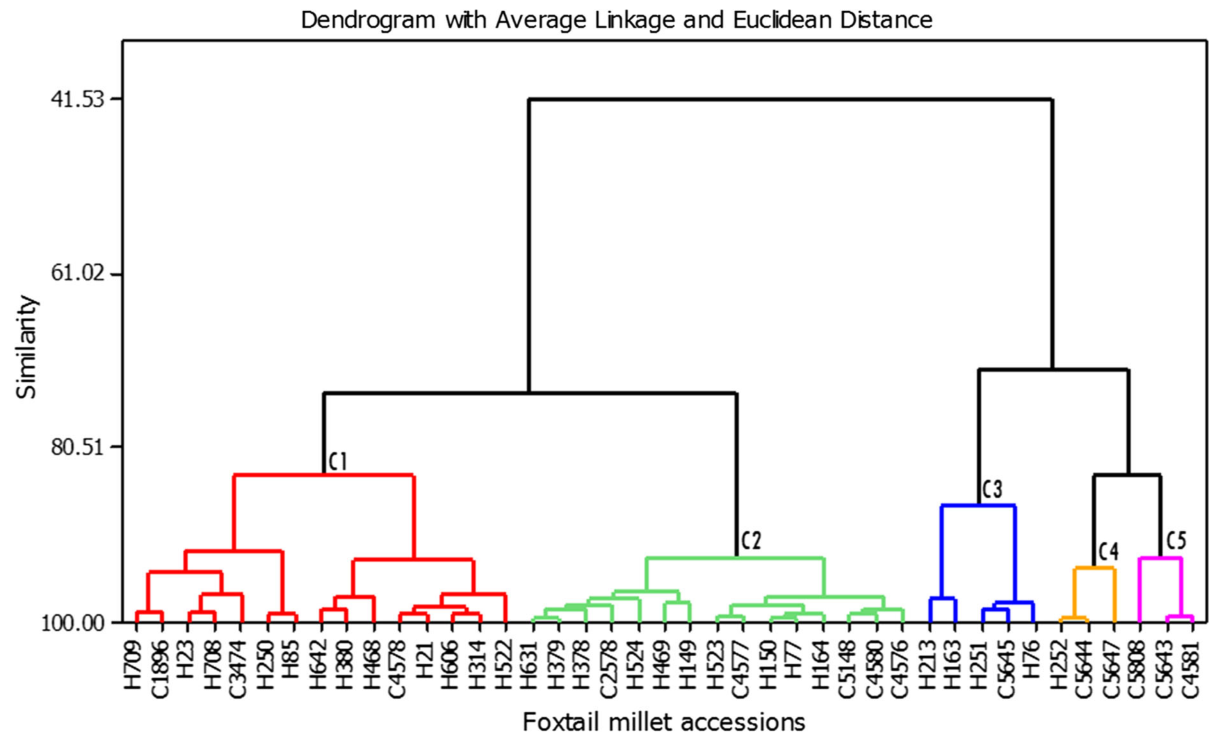

Fig. 3 UPGMA clustering of 41 foxtail millet landraces based on Euclidean distance

Table 4 Number of accessions with average of major agronomic traits in each cluster

Variable	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5
Accessions from Humla	12	11	4	1	–
Accessions from Jumla	2	3	–	–	1
Accessions from Lamjung	–	–	1	2	1
Accessions from Gorkha	–	–	–	–	1
Accessions from Kavre	–	1	–	–	–
Accessions from Bajhang	1	–	–	–	–
Number accessions	15	15	5	3	3
Days to heading	60	60	62	61	57
Days to maturity	97	102	97	92	86
Plant height (cm)	157	142	197	180	164
Panicle length (cm)	22	22	20	23	20
Panicle width (mm)	16.3	15.8	20.8	27.7	25.7
Panicle exertion (cm)	16	14	18	14	18
Grain yield (kg/ha)	1150	279	2174	3416	2856

accessions. The first five principal components accounted for 32, 28, 15, 11 and 9%, respectively of the total phenotypic variance among accessions based on 7 quantitative traits. Scatter plot of the first two principal components, accounting 60% of cumulative variance, suggested that PCA is in support with the result of cluster analysis (Fig. 4).

Correlation analysis

Pearson's correlation coefficient between quantitative traits has been presented in Table 6. There was highly significant positive correlation between days to heading and days to maturity. Significant but negative correlation was observed between days to heading and

Table 5 Principal component analysis and Eigen analysis of the correlation matrix

Variables	PC1	PC2	PC3	PC4	PC5
Eigen value	2.24	1.93	1.08	0.77	0.61
Proportion	0.32	0.28	0.15	0.11	0.09
Cumulative	0.32	0.60	0.75	0.86	0.95
Days to heading	0.164	− 0.639	0.087	− 0.184	0.137
Days to maturity	0.398	− 0.359	0.374	− 0.217	− 0.487
Plant height	− 0.435	− 0.255	0.237	− 0.532	0.479
Panicle length	− 0.216	0.496	0.157	− 0.622	− 0.319
Panicle width	− 0.486	− 0.29	− 0.109	0.06	− 0.641
Panicle exertion	− 0.068	0.169	0.871	0.363	0.042
Grain yield	− 0.581	− 0.197	0.034	0.337	− 0.003

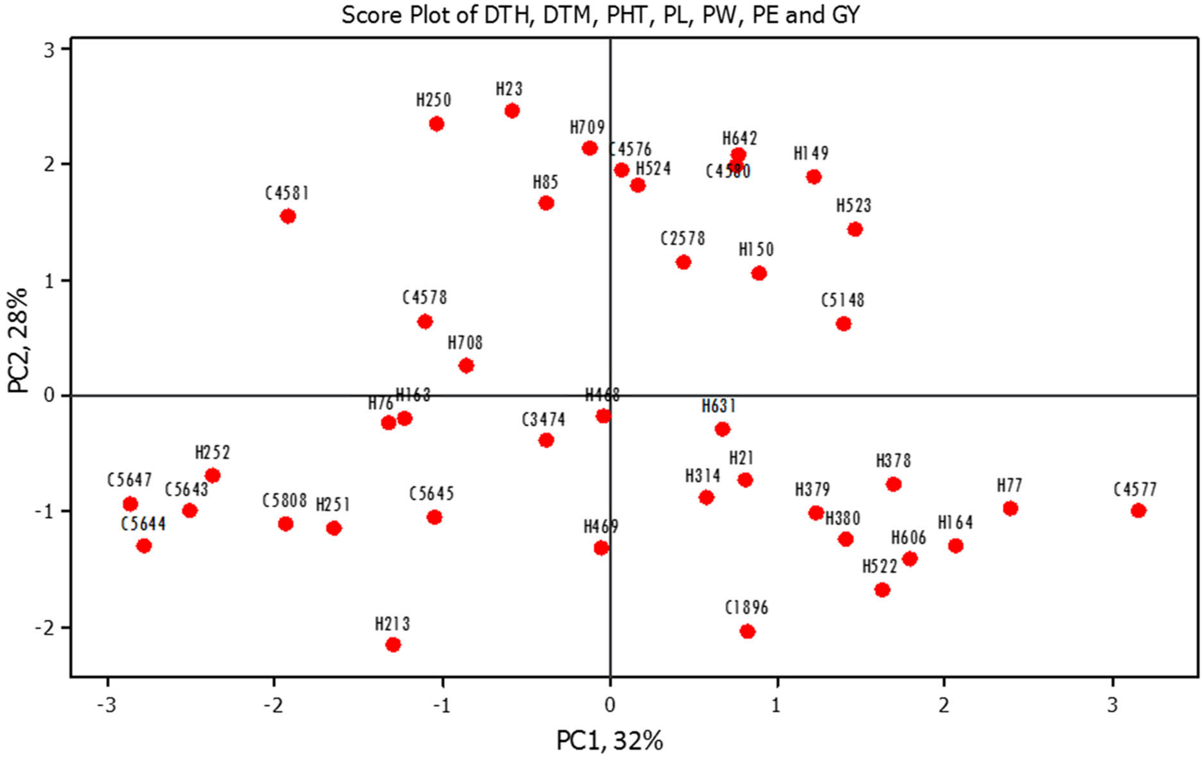

Fig. 4 Scatter plots of foxtail millet landraces based on first two principal components

panicle length. Similarly days to maturity was negatively correlated with grain yield suggested that early genotypes gave higher yield as an adaptive trait under semi-arid and cold environment, thus early maturing accessions can be selected for dry areas which can escape from terminal drought and cold stresses. Plant height showed highly significant positive correlations with grain yield and panicle width, respectively suggesting that taller height can be considered as a desirable character of high yielding accession. The strong association between grain yield and panicle width suggested that accessions with thicker panicles can be selected as a high yielding trait.

Crop genetic diversity of traditional mountain crops such as foxtail millet has disappeared in many parts of Himalayan mountains (Sheikh and Singh 2013). Community richness of foxtail millet diversity in Lamjung, Humla and Jumla has shown declining trend

Table 6 Pearson's correlation analysis between 7 quantitative traits

Trait	Days to heading	Days to maturity	Plant height	Panicle length	Panicle width	Panicle exertion
Days to maturity (days)	0.544**					
Plant height (cm)	0.240	− 0.141				
Panicle length (cm)	− 0.568**	− 0.297	0.137			
Panicle width (mm)	0.121	− 0.131	0.383*	0.024		
Panicle exertion (cm)	− 0.173	0.068	0.060	0.165	− 0.101	
Grain yield (kg/ha)	− 0.028	− 0.378*	0.522**	− 0.044	0.700**	0.122

in recent decades. Recent surveys indicated area under foxtail millet and the number of households growing the crop is shrinking in mountains of Nepal (Gurung et al. 2016; Parajuli et al. 2017; Palikhey et al. 2016). Local crop genetic diversity from these areas exists in National Genebank or in farmers' fields but they are not characterized and evaluated. They are not easily accessible to majority of farmers. This is not surprising as globally the use of ex situ materials is limited by lack of characterization data and information (Hodgkin et al. 2003) and direct use of genebank materials by re-introduction by farmers may benefits farming community immediately amongst less researched crops. With increased awareness on nutritional and health benefits and climate resilient properties of the crop, foxtail millet research for development in low HDI index districts of Nepal is recently recognized. Farmers of these areas of Nepal suffer from food insecurity, malnutrition and hidden hunger and therefore, importance of traditional crops like foxtail millet remains important. PRA surveys suggest that farmers prefer varieties have set of traits such as higher yield, earliness, easy threshing and taste. Participatory methods proposed by Joshi and Sthapit (1990) and Witcombe et al. (2016) will be employed for further evaluation in diverse geographic environments. The application of this study will be used to distribute a set of IRD kits i.e. half kg seed per HH of six landraces (namely C5644, H252, C5647, C5808, C5643 and C4581) in a range of 500–1000 kits for deploying new diversity and provide farmers for participatory selection for their needs. The pre-breeding or grassroots breeding of the crop will help to provide better options than available in local market (Sthapit and Ramanatha Rao 2009) and the best way in which genebank diversity can be used as a strategy for such nutritionally dense and climate resilient neglected crops, will have considerable positive effect on use of under-utilized ex situ genetic resources (Goron and Raizada 2015).

Conclusion

Among the 41 accessions of foxtail millet collected from six districts of the country, high level of diversity was observed for different agro-morphological markers like growth habit, phenology, plant stature, length and width of inflorescence, flag leaf angle, leaf and sheath pubescence, leaf blast and stem borer susceptibility, grain yield and grain colour, etc. High grain yield was associated with earliness, tallness and thicker panicles. Accessions C5644, H252, C5647, C5808, C5643 and C4581 are the important landraces (three from Lamjung and one each from Gorkha, Humla and Jumla) needs further evaluation in farmers' field of multiple locations to identify candidate genotype for direct cultivation and also for future use in crop improvement programmes. The project aims to test these materials in large number (500–1000 kits) in wide geographic region of Nepal using participatory diversity kits for deploying new diversity and make traditional seed system more resilient. Farmers now have a greater range of genetic diversity available from genebank to them than ever before and have flexibility for local selection for target environment.

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Compliance with ethical standards

Conflicts of interest There are no conflicts of interests to be declared.

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