Diversity sourcing of foxtail millet through diversity assessment and on-farm evaluation

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Abstract: Drawing upon the serious food insecurity issues and continually growing impacts of climate change on livelihood, diversity sourcing of climate-resilient, nutritionally rich crops like foxtail millet through diversity assessment and on-farm evaluation could be a reliable avenue to meet farmer’s need and improve food security in the extremely mountainous agro-ecology of Nepal and elsewhere. In context of meager research on foxtail millet in Nepal, we conducted on-farm diversity assessment studies of 27 Nepalese foxtail millet landraces in 2015. Subsequently, we identified 8 locally adaptable and robust foxtail millet genotypes based on the inferences of diversity block trial and evaluated them under Humla and Jumla conditions in 2016 for sourcing end-user preferred varietal diversity. Our studies revealed existence of marked diversity among the Nepalese foxtail millet accessions, which could be effectively utilized for crop improvement. Kalo Kaguno, Seto Kaguno, Aule Kaguno, and CO1896 were found to be superior yielders in descending order while CO5644 and CO5647 were substantially found early maturing. Apart from acknowledging the potentiality of these genotypes as promising parents, we encourage deployment of the sourced diversity through participatory plant breeding approaches.

ABOUT THE AUTHOR
Ritesh Kumar Yadav, Achyut Raj Adhikari, and Subash Gautam are young and energetic researchers with major research emphasis on genetic diversity assessment and evaluation of crop which yet remains neglected and under-utilized despite its tremendous potentialities in climate change adaptation and resilience, participatory methods to assess and use agricultural biodiversity, participatory crop improvement and in situ and on-farm conservation. Krishna Hari Ghimire is a senior scientist who is highly experienced in agro-biodiversity conservation, management and utilization, conventional as well as molecular plant breeding approaches, and participatory crop improvement. Rajeev Dhakal is a plant breeder who seeks to develop a high-yielding, stable and farmer-preferred varieties of crops of future such as millets, amaranth, buckwheat, cold-tolerant rice, and barley through molecular and participatory plant breeding approaches.

PUBLIC INTEREST STATEMENT
Foxtail millet is a traditional, climate-resilient and highly nutritious crop of the high mountains of Nepal. Despite its unrivaled potential in climate change adaptation and food security, it yet remains neglected and under-utilized and very meager attention has been given by the public and private plant breeding sector for crop improvement and formal seed distribution system. No any foxtail millet has been released in Nepal to the date. The present study was conducted on diversity sourcing of promising genotypes from ex situ and on farm hinged on diversity assessment and evaluation studies. The findings suggested tremendous diversity among the studied landraces. Identification and diversity sourcing of high-yielding and farmer-preferred genotypes complemented the robust breeding program. Our findings encourage deployment of the promising sourced diversity through participatory plant breeding approaches to expand and promote the varietal choice options for expeditious benefits to the local farmers.
participatory plant breeding approaches using informal research and development and diversity kits for expeditious benefits to the local farmers.

**Subjects:** Agronomy; climate change adaptation; food security; neglected and under-utilized crop; on-farm research; plant breeding

**Keywords:** diversity sourcing; food security; foxtail millet; Nepal; on-farm evaluation

1. **Introduction**

Foxtail millet (*Setaria italica* (L.) Beauv.) (Vernacular name: Kaguno/Kanguno) is underutilized and neglected traditional crop that is hardy and can grow well in rainfed and marginal lands of high mountains of Nepal (Singh, Bahunga, & Bhatt, 2015), making them the preferred cereal crop for drought-prone areas. It is a self-pollinating crop with chromosome number, \(2n = 18\), classified under the family Poaceae and subfamily Panicoideae (Fedorov, 1974). It is though to be indigenous to southern Asia and considered one of the oldest cultivated millets (Oelke et al., 1990). It has longest history of cultivation among the millets having grown in China for about 8,000 years. It was cultivated as an important food across southern Europe until the early twentieth century (Austin, 2006). It ranks second among the millets across the globe in terms of production and is cultivated in around 26 countries. In Nepal, it is mostly cultivated in marginal and non-irrigated condition of mid and high hills, particularly the Karnali region. Major foxtail millet growing districts in Nepal are Mugu, Kalikot, Humla, Jumla, Bajhang, Dolpa, Lamjung, Gorkha, Ramechhap, Kavre, etc., where crop is grown sole as well as inter-cropped with finger millet (*Ekeusine coracana* Gaertn.), proso millet (*Panicum miliaucum* L.), beans (*Phaseolus vulgaris*), amaranths (*Amaranthus hypochondriatus*), maize (*Zea mays* L.), etc. (Ghimire, Joshi, Gurung, & Sthapit, 2018). This crop is associated with traditional values and food culture of mid-hills and high hills of Nepal as well. Grains of foxtail millet are used to prepare regular staple diet *bhat* (steamed rice) and *khir* (pudding) while *Dhido* (porridge), *thukpa* (Tibetan noodle), *laddu* (Nepali sweet), and *puwa* (kind of pancake) are prepared from its flour. It is also used in fermented form as *chhyang* and *chhyangkhol* (local liquors) by different ethnic groups of Karnali region. The crop has tremendous utility and application in baking and noodle industries. It is also recently acknowledged because of health and medicinal benefits such as reducing blood glucose levels and cholesterol control in normal as well as diabetic patients (Ghimire et al., 2018; Kamatar et al., 2014a; Kamatar et al., 2014b). Apart from its high nutritional and medicinal value, it exhibits high photosynthetic efficiency and drought tolerance under low fertility conditions (Dai et al., 2008, 2011). For its incomparable adaption in low fertility and drought conditions, foxtail millet is widely adapted to mountainous areas of Nepal.

Nepal is one of the centers of diversity of foxtail millet (Nakayama, Namai, & Okuno, 1999) and has high genetic diversity of this crop (Mo FSC, 2002). In Nepal, this crop is considered as traditional climate resilient and nutritionally dense crop but trends of cultivation and use are reported to be shrinking fast due to globalization, land-use change, out migration, social values, change in food habit, depleting traditional knowledge, and last but not the least, lack of research and policy support such as crop improvement and formal seed distribution system (Bisht et al., 2006; Gurung, Sthapit, Gaucho, Joshi, & Sthapit, 2016; Palikhey et al., 2016; Parajuli et al., 2017; Sheikh & Singh, 2013). The study of genetic diversity and genetic relatedness is necessary for crop improvement and in developing appropriate strategies for the conservation, exploitation, and utilization of foxtail millet accession (Upadhyya & Joshi, 2003). Still, very few studies on characterization and evaluation for wider use have been conducted due to lack of interest and priority of public and private breeding programs in foxtail millet.

In the wake of continually bulging climate change consequences, food and nutritional insecurity in the remote and fragile mountainous areas, foxtail millet is an unrivaled climate-resilient and future crop that needs to be revitalized and emphasized in agricultural and public plant breeding programs for its adaptation to marginal soil conditions and environmental
stresses, particularly drought, nutritional benefits, and medicinal benefits. Realizing the tremendous untapped potential of the crop (Goron & Raizada, 2015), very meager foxtail millet studies, and state of no any foxtail millet variety registered/released and available in the national agricultural system of Nepal, the present study was conducted to: (1) dissect the agro-morphological diversity among the Nepalese foxtail millet accessions, (2) to identify and evaluate promising genotypes for diversity sourcing and diversity deployment, and (3) to determine the association between the studied traits and principal traits contributing to total variation. The present study would provide useful information to facilitate the choice of genitors for foxtail millet breeding programs, source agronomically superior diversity and diversify varietal selection choices for expeditious benefits to the farmers and ultimately, enable rapid release and registration of robust genotypes in national agricultural system.

2. Materials and methods

2.1. Experimental materials and design

The experiments were conducted during 2015 and 2016 under on-farm conditions in Hanku village development committee (VDC) of Jumla district (N 29° 14.983′ E 82° 06.638′, altitude 2274 m) and Chhipra VDC of Humla district (N 29° 55.833′, E 81° 51.843′, altitude 2197 m). Twenty-seven accessions (all landraces) of foxtail millet collected from ex situ and on farm were included in the study for their agro-morphological characterization and diversity assessment in 2015 (Table 1). The Diversity Block was set up in Hanku VDC of Jumla. Diversity block is an experimental block of farmers’ varieties managed by local institution for research and development purposes (Tiwari et al., 2006). 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. Characterization was performed on the basis of four quantitative traits (plant height, panicle exertion, panicle length, and flag leaf length) and five qualitative traits (grain yield potential, flag leaf angle, leaf sheath color, leaf pubescence, and overall phenotypic acceptance) as per the descriptors of S. italica (L.) Beauv. After subjecting the data to cluster analysis, the candidate genotypes from cluster showing eminence in those traits were selected for their inclusion in yield trial. In 2016, total of eight (seven selected from the diversity block trial and one promising accession from the national agricultural research system) were chosen for their further evaluation of agronomic traits (Table 2). The yield trial experiment was laid out in a randomized block design with three replications in Humla and Jumla. In each replication, each cultivar was grown in a plot of 10 rows with a distance of 20 cm between row to row and 10 cm between plant to plant and the area of each experimental unit was 4.8 m$^2$ (2 m × 2.4 m). Sowing of each genotype was done on 2 May 2016. The local farmer’s practices were followed to raise the crop. Well rotten farm yard manure at the rate of 4 t ha$^{-1}$ was applied in the field during land preparation. No chemical fertilizers, insecticide, or pesticide were used. Two hand weeding and hoeing were carried out at 30 and 65 days after sowing. The trial was conducted in irrigated condition. The data were recorded for six agro-morphological traits such as days to 50% heading, days to 80% maturity, plant height (cm) at maturity, panicle length (cm) at maturity, grain yield (kg plot$^{-1}$), and thousand grain weight (g). The grain yield (kg plot$^{-1}$) was later extrapolated to t ha$^{-1}$. The genotypes included in the study along with their respective sources are presented in Tables 1 and 2, respectively.

2.2. Data collection and analysis

The data collected for agro-morphological characterization of the 27 genotypes included in the diversity block during 2015 were subjected to cluster analysis, based on Euclidean distance. The descriptive statistics for studied traits of each cluster were also computed. Shannon Weaver Diversity indices were calculated in order to estimate the phenotypic diversity for each quantitative and qualitative trait with Microsoft Excel using the formula: Standard $H' = \sum (n/k) \log_2(n/k)$, 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
Table 1. List of foxtail millet landraces used in diversity block along with their source

| Entry | Accession no./Local name | District | Source       |
|-------|--------------------------|----------|--------------|
| 1     | CO1896                   | Jumla    | NAGRC*       |
| 2     | CO2578                   | Kabre    | NAGRC        |
| 3     | CO4576                   | Jumla    | NAGRC        |
| 4     | CO4577                   | Jumla    | NAGRC        |
| 5     | CO5148                   | Humla    | NAGRC        |
| 6     | CO4580                   | Jumla    | NAGRC        |
| 7     | Humla-DF-149 (Rato Kaguno)| Humla    | NAGRC        |
| 8     | Humla-DF-150 (Kalo Kaguno)| Humla    | NAGRC        |
| 9     | Humla-DF-164 (Pahenlo Kaguno) | Humla    | NAGRC |
| 10     | Humla-DF-21 (Kalo Kaguno) | Humla    | NAGRC        |
| 11     | Humla-DF-314 (Rato Kaguno) | Humla    | NAGRC        |
| 12     | Humla-DF-379 (Kalo Kaguno) | Humla    | NAGRC        |
| 13     | Humla-DF-380 (Kalo Kaguno) | Humla    | NAGRC        |
| 14     | Humla-DF-522 (Seto Kaguno) | Humla    | NAGRC        |
| 15     | Humla-DF-523 (Rato Kaguno) | Humla    | NAGRC        |
| 16     | Humla-DF-606 (Kalo Kaguno) | Humla    | NAGRC        |
| 17     | L1 Kalo                  | Lamjung  | NAGRC        |
| 18     | L2 Rato (Rato Kaguno)    | Lamjung  | NAGRC        |
| 19     | L3 (Tinmase)             | Lamjung  | NAGRC        |
| 20     | L4 (Ande)                | Lamjung  | NAGRC        |
| 21     | L5 (Bariyo)              | Lamjung  | NAGRC        |
| 22     | L6 (Seto)                | Lamjung  | NAGRC        |
| 23     | L7 (Parbeli)             | Lamjung  | NAGRC        |
| 24     | J1 (Sthaniya Rato)       | Jumla    | NAGRC        |
| 25     | J2 Rato                  | Jumla    | NAGRC        |
| 26     | J3 (Pahelo Seto)         | Jumla    | NAGRC        |
| 27     | Aule Kaguno (Jumla)      | Jumla    | NAGRC        |

*National Agriculture Genetic Resources Centre, Khumaltar, Nepal.

Table 2. List of foxtail millet genotypes included in yield trial

| S.N. | Genotypes                  | Source       |
|------|----------------------------|--------------|
| 1    | Pahela Kaguno (Humla)      | NAGRC*       |
| 2    | CO5647 (Lamjung)           | NAGRC        |
| 3    | CO1896 (Jumla)             | NAGRC        |
| 4    | Kalo Kaguno (Humla)        | NAGRC        |
| 5    | CO5644 (Lamjung)           | NAGRC        |
| 6    | Aule Kaguno (Jumla)        | Jumla        |
| 7    | Rato Kaguno (Jumla)        | Jumla        |
| 8    | Seto Kaguno (Humla)        | Humla        |

*National Agricultural Genetic Resources Centre, Khumaltar, Nepal.
number of observations for that character. For multi-location yield trials in Humla and Jumla, combined analysis of variance under randomized complete block design using statistical software R and the significance of differences between the means were compared using least significant difference (LSD) at 5% level of significance. The Pearson’s correlation coefficients between each pair of traits and principal component analysis (PCA) were conducted using statistical software R and Minitab 15.0 in order to study the extent of association among the traits and to identify the patterns of agro-morphological variation, respectively.

3. Results and discussion

3.1. Experiment I: diversity block (2015)

Six distinct clusters were revealed upon cluster analysis of twenty-seven genotypes by traits viz., panicle exertion, panicle length, grain yield potential and overall phenotypic acceptance based on average Euclidean distance (Figure 1). The descriptive statistics of distinct clusters are presented in Table 3. The number of genotypes belonging to distinct clusters varied from 1 in Cluster VI to 13 in Cluster IV. Cluster IV was the largest cluster comprising 13 genotypes (48.15%) followed by Clusters III and V with 4 genotypes in each. Pahelo Kaguno (Humla) and Seto Kaguno (Humla) were selected from this cluster for further evaluation in yield trial based on the convincing yield potential, panicle length, panicle exertion, and overall phenotypic acceptance. Rato Kaguno (Lamjung), Kalo Kaguno (Humla), and Rato Kaguno (Jumla) from Cluster V were selected for yield trial based on their superiority in terms of grain yield potential and panicle exertion. Members of Cluster I, Aule Kaguno (Jumla) and CO1896 (Jumla) were selected for yield trial in 2016 considering its larger panicle length and grain yield potential. Similarly, CO5644 was selected from the National gene bank pool based on its performance in on-station trial. The cluster analysis also revealed that the genotype aggregation was independent of the geographic source of collection. Characterization of accessions and clustering based on their morphological and genetic similarity aids in identification and selection of the genitors which could be effectively utilized in hybridization programs. Hence, grouping of landraces using unweighted pair group method with arithmetic mean (UPGMA) cluster analysis would be valuable for the breeders in such a way that the most promising genotypes in the population may be selected from different clusters for crop improvement.

Descriptor states and their frequency as well as Shannon–Weaver diversity indices for each quantitative and qualitative traits are depicted in Tables 4 and 5. The Shannon–Weaver index ($H'$) for quantitative traits ranged from 0.74 to 0.80 (Table 4) while for qualitative traits, 0.71 to unity...
Table 3. Means values and range of decisive agro-morphological traits within clusters of foxtailmillet accessions

| Cluster | Members | Panicle exertion (cm) | Panicle length (cm) | Grain yield potential (3=low, 5=intermediate, 7=high) | Overall phenotypic acceptance (1-excellent, 5-intermediate, 9-unacceptable) |
|---------|---------|-----------------------|---------------------|-------------------------------------------------------|--------------------------------------------------------------------------|
| I       | CO1896, Aule (Jumla), CO4577 (3) | 12.67 (10.00-14.02)  | 19 (16-21)          | 5 (3-6)                                               | 6.34 (5-8)                                                              |
| II      | CO2578, L3 (Tinmase) (2)          | 16 (15-17)           | 17 (16-18)          | 3                                                   | 5 (4-6)                                                                  |
| III     | CO4576, Humla-DF-150, Humla-DF-149, CO5148 (4) | 21 (19.80-22)   | 13.5 (12.52-16.70)  | 3.5 (2-5)                                             | 2.75 (1-6)                                                              |
| IV      | CO4580, Humla-DF-164, Humla-DF-522, Humla-DF-379, Humla-DF-380, L5 (Bariyo), Humla-DF-606, L1 (Kalo), J1 (Sthaniya Rato), J2 (Rato), L4 (Ande), L7 (Parbeli) (13) | 15.77 (14.32-28.32) | 12.54 (11.32-16.58) | 4.69 (4-6)                                           | 4.37 (3-6)                                                              |
| V       | Humla-DF-21, L2 (Rato), Humla-DF-314, Humla-DF-523 (4) | 17 (13-21)          | 15.5 (14-19)        | 7 (5-7)                                               | 6.25 (5-8)                                                              |
| VI      | J3 (Pahela Seto) (1)              | 11.23                | 12.76               | 7                                                     | 7                                                                        |

Figures in parenthesis represent the range of values of traits for each cluster.
Figures in bold in the curly bracket represent the number of members within each cluster.
Table 4. Descriptive statistics and Shannon–Weaver index of quantitative traits of foxtail millet accessions

| Quantitative traits       | Shannon–Weaver $H'$ | Mean ± S.E. | Minimum | Maximum |
|---------------------------|---------------------|-------------|---------|---------|
| Plant height (cm)         | 0.74                | 133.7 ± 3.00| 94      | 162     |
| Panicle exertion (cm)     | 0.76                | 16.2 ± 0.60 | 10      | 22      |
| Panicle length (cm)       | 0.80                | 14 ± 0.50   | 11      | 21      |
| Flag leaf length (cm)     | 0.80                | 21.4 ± 0.77 | 16.1    | 32.1    |

Table 5. Descriptor states and Shannon–Weaver index of qualitative traits of foxtail millet accessions

| Qualitative traits        | $H'$  | Descriptor’s states | Frequency | Proportion (%) |
|---------------------------|-------|---------------------|-----------|----------------|
| Grain yield potential     | 0.90  | Low                 | 7         | 25.93          |
|                           |       | Intermediate        | 15        | 55.56          |
|                           |       | High                | 5         | 18.52          |
| Flag leaf angle           | 1.00  | Erect               | 7         | 25.93          |
|                           |       | Intermediate        | 7         | 25.93          |
|                           |       | Horizontal          | 7         | 25.93          |
|                           |       | Descending          | 6         | 22.22          |
| Leaf sheath color         | 0.71  | Green               | 15        | 55.56          |
|                           |       | Slightly pale red   | 2         | 7.41           |
|                           |       | Pale red            | 4         | 14.81          |
|                           |       | Red                 | 6         | 22.22          |
| Leaf pubescence           | 0.88  | Dense               | 4         | 14.81          |
|                           |       | Medium              | 8         | 29.63          |
|                           |       | Sparse              | 15        | 55.56          |
| Overall phenotypic        | 0.78  | 1                    | 0          | 0.00           |
| acceptance                |       | 2                    | 3          | 11.11          |
|                           |       | 3                    | 2          | 7.41           |
|                           |       | 4                    | 7          | 25.93          |
|                           |       | 5                    | 6          | 22.22          |
|                           |       | 6                    | 5          | 18.52          |
|                           |       | 7                    | 4          | 14.81          |
|                           |       | 8                    | 0          | 0.00           |
|                           |       | 9                    | 0          | 0.00           |

(Table 5), suggesting existence of colossal diversity among the 27 foxtail millet genotypes for recorded traits with varying degrees of contribution to the phenotypic diversity. Plant height ranged from 94 to 162 cm with average of 133.7 cm which is quite taller than the Nepalese collection (70–150 cm) but shorter than the global collection (20–215 cm) (Reddy et al., 2006) and that (108–232 cm) reported by Ghimire et al. (2018). Our foxtail millet collections showed narrower range of panicle exertion (10–22 cm) as compared to the range in the global collection of 1–36 cm and the Indian collection (Reddy et al., 2006) and similar to the range of 7–30 cm reported by Ghimire et al. (2018). Panicle length ranged from 11 to 21 cm which is narrower than the range of
| Genotypes          | Days to heading | Days to maturity | Plant height (cm) | Panicle length (cm) | Grain yield (t ha\(^{-1}\)) | 1000-grain weight (g) |
|--------------------|-----------------|------------------|-------------------|---------------------|----------------------------|-----------------------|
| Aule Kaguno        | 96.00 ± 1.53    | 139.33 ± 0.67c   | 251.87 ± 9.48ab   | 24.67 ± 1.75bc      | 3.041 ± 0.58bcd            | 2.00 ± 0.00c          |
| CO1896(Jumla)     | 89.33 ± 8.17    | 143.00 ± 0.58b   | 232.87 ± 9.83c    | 21.13 ± 0.71cde     | 3.44 ± 0.06abc            | 2.93 ± 0.07a          |
| CO5644(Lamjung)   | 89.33 ± 6.67    | 115.00 ± 0.00e   | 235.87 ± 7.35bc   | 17.79 ± 0.91e       | 3.08 ± 0.29bcd            | 2.07 ± 0.07c          |
| CO5647(Lamjung)   | 84.00 ± 6.66    | 119.00 ± 0.58d   | 235.00 ± 6.66bc   | 19.80 ± 1.62de      | 2.71 ± 0.17cd            | 2.13 ± 0.07c          |
| Kalo Kaguno       | 90.67 ± 4.48    | 142.00 ± 0.00b   | 261.20 ± 1.50a    | 25.67 ± 1.07ab      | 4.13 ± 0.19a             | 3.00 ± 0.00a          |
| Pahelo Kaguno     | 88.33 ± 4.70    | 142.33 ± 1.20b   | 259.87 ± 3.45a    | 24.20 ± 1.70bcd     | 2.40 ± 0.40d             | 2.93 ± 0.07a          |
| Rato Kaguno       | 89.67 ± 8.88    | 151.33 ± 0.67a   | 254.73 ± 8.18a    | 29.80 ± 0.76a       | 2.25 ± 0.06d             | 2.93 ± 0.07a          |
| Seto Kaguno       | 77.67 ± 6.17    | 139.33 ± 0.67c   | 268.00 ± 5.46a    | 25.33 ± 2.15bc      | 3.79 ± 0.15ob            | 2.37 ± 0.03b          |
| F-value           | 0.70            | 434.76           | 5.13              | 6.73                | 4.78                       | 67.00                  |
| F-test            | NS              | **               | **                | **                  | **                        | **                    |
| p-value           | 0.6749          | <0.00001         | 0.0046            | 0.0013              | 0.0063                     | <0.00001              |
| S.Em:             | 9.06            | 0.85             | 8.48              | 2.08                | 0.20                       | 0.77                  |
| LSD (p ≤ 0.05)    | -               | 1.83             | 18.19             | 4.45                | 0.44                       | 0.16                  |
| CV (%)            | 12.60           | 0.77             | 4.16              | 10.79               | 16.71                      | 3.70                  |

**Highly significant (p ≤ 0.01), *significant (p ≤ 0.05).**
Mean values in columns with different letters are significantly different (p ≤ 0.05) according to Fisher’s LSD test.
| Genotypes          | Days to heading | Days to maturity | Plant height (cm) | Panicle length (cm) | Grain yield (t ha⁻¹) | 1000-grain weight (g) | F-value | F-test | p-value |
|--------------------|----------------|-----------------|-------------------|---------------------|----------------------|-----------------------|---------|-------|---------|
| Aule Kaguno        | 102 ± 0.00c    | 131.00 ± 1.53d  | 161.73 ± 8.25bc   | 18.20 ± 0.76        | 2.83 ± 0.27ab        | 2.57 ± 0.09a          |         |       | <0.00001|
| CO1896(Jumla)      | 121.00 ± 0.00a | 159.67 ± 4.70a  | 179.33 ± 9.50a    | 18.93 ± 0.41        | 2.15 ± 0.17cd        | 2.17 ± 0.02b          | **      | **    | <0.00001|
| CO5644(Lamjung)    | 107.33 ± 1.33b | 13.00 ± 1.00c   | 139.93 ± 2.31d    | 18.87 ± 0.52        | 1.85 ± 0.06d         | 1.73 ± 0.05c          |         |       | 0.0016  |
| CO5647(Lamjung)    | 107.33 ± 1.33b | 138.67 ± 0.88c  | 162.20 ± 3.14bc   | 17.60 ± 0.23        | 2.16 ± 0.15cd        | 1.79 ± 0.08c          |         |       | 0.0016  |
| Kala Kaguno        | 107.33 ± 1.33b | 159.00 ± 0.00a  | 179.27 ± 6.69a    | 21.20 ± 0.20        | 3.02 ± 0.29a         | 2.28 ± 0.07b          |         |       | 0.0016  |
| Pahelo Kaguno      | 108.00 ± 1.53b | 152.33 ± 0.67b  | 176.20 ± 5.05ab   | 19.73 ± 1.64        | 2.71 ± 0.06abc       | 2.28 ± 0.04b          |         |       | 0.0016  |
| Rato Kaguno        | 97.00 ± 0.00d  | 129.00 ± 0.00d  | 155.73 ± 7.13cd   | 17.40 ± 1.86        | 2.25 ± 0.38bcd       | 2.67 ± 0.13a          |         |       | 0.0016  |
| Seto Kaguno        | 104.00 ± 0.00c | 136.67 ± 0.33c  | 157.47 ± 3.24c    | 17.60 ± 0.42        | 2.58 ± 0.16abc       | 2.53 ± 0.02a          |         |       | 0.0016  |

**Highly significant (p ≤ 0.01), *significant (p ≤ 0.05).**

Mean values in columns with different letters are significantly different (p ≤ 0.05) according to Fisher’s LSD test.
2–39 cm in the Indian Collection (Reddy et al., 2006) and range in the Nepalese collection of 13–25 cm and 13–30 cm (Ghimire et al., 2018; Reddy et al., 2006). Similarly, flag leaf length ranged from 16.1 cm to 32.1 cm and showed more or less similar range in the Indian collection (21.08–35.44 cm) reported by Geethanjali and Jegadeeswaran (2016).

3.2. Experiment II: yield trial (2016)
The combined analysis of variance for the studied agro-morphological traits in yield trial revealed significant differences among the test genotypes and environments (Table 8). The variance of genotype was highly significant (p ≤ 0.01) for all the studied traits except days to 50% heading. Highly significant mean squares (p ≤ 0.01) for days to 80% maturity, plant height, panicle length, and 1000-grain weight and significant mean square for grain yield due to genotype x environment interaction revealed that the genotypes responded differently to different environmental conditions, confirming the importance of agro-morphological characterization of the foxtail millet genotype in multi-locations. In addition, the variation due to environment was found highly significant for all the studied traits. The mean performance of the genotypes for the studied traits in Humla and Jumla are depicted in Tables 6 and 7, respectively. In Jumla conditions, CO1896 (Jumla) recorded the highest number of days to heading (121.00 ± 0.00) while Rato Kaguno recorded the lowest value (97.00 ± 0.00) for days to heading. Rato Kaguno and Kalo Kaguno recorded the highest value for days to 80% maturity in Humla and Jumla, respectively. Similarly, the lowest value for days to 80% maturity was recorded by CO5644 (115.00 ± 0.00) and Rato Kaguno (129.00 ± 0.00) being at par with Aule Kaguno (131.00 ± 1.53) in Humla and Jumla, respectively. The highest plant height was recorded by Seto Kaguno (268.00 ± 5.46 cm) statistically being at par with Rato Kaguno and Pahelo Kaguno in Humla and CO1896 (179.33 ± 9.50 cm) being at par with Kalo Kaguno. Rato Kaguno being at par with Kalo Kaguno recorded the highest panicle length in Humla while the variation for panicle length was non-significant in Jumla. Kalo Kaguno was found to be superior yielder in both environmental conditions by recording grain yield of 4.13 ± 0.19 and 3.02 ± 0.29 t ha⁻¹ in Humla and Jumla, respectively. Kalo Kaguno recorded the highest 1000-grain weight (3.00 ± 0.00 g) in Humla being at par with Pahelo Kaguno and Rato Kaguno in Humla while Rato Kaguno (2.67 ± 0.13 g) being statistically at par Aule Kaguno and Seto Kaguno recorded highest 1000-grain weight in Jumla conditions. Combined analysis of variance revealed significant to highly significant differences for location, genotype, and genotype x location for all the studied traits except days to heading (Table 8). The pooled mean performance of the genotypes for the studied traits over the two locations is presented in Table 9. CO1896 recorded the highest pooled mean (151.33 ± 4.29) for days to maturity being at par with Kalo

| Source of variance | Degree of freedom | Mean sum of square |
|--------------------|-------------------|--------------------|
|                     |                   | Days to heading    | Days to maturity | Plant height | Panicle length | Grain yield | 1000-grain weight |
| **Genotype (G)**    | 7                 | 111.045**          | 536.76**          | 655.97**     | 23.36**       | 5.88**     | 0.6352**         |
| **Location (L)**    | 1                 | 4162.69**          | 526.69**          | 88374.00**   | 283.14**      | 25.93**    | 1.0384**         |
| **G x L**           | 7                 | 116.54**           | 387.16**          | 468.58**     | 25.1473**     | 25.93**    | 0.3175**         |
| LSD (p ≤ 0.05)      |                   | 2.84               | 11.42             | 2.48         | 0.25          | 0.13       | 0.13             |
| LSD (p ≤ 0.01)      |                   | 4.62               | 1.42              | 5.71         | 1.24          | 0.13       | 0.07             |
| LSD G x E (p ≤ 0.05) |                   | 4.02               | 16.15             | 3.50         | 0.36          | 0.19       |                  |
| CV (%)              |                   | 8.04               | 1.73              | 4.68         | 9.95          | 16.08      | 4.68             |
| MSE                 |                   | 61.3792            | 5.8097            | 93.8457      | 4.4162        | 0.0459     | 0.0126           |

**Highly significant (p ≤ 0.01), *significant (p ≤ 0.05), ns non-significant, MSE Mean square of Error.**
## Table 9. Pooled mean ± S.E. of different agro-morphological traits of foxtail millet genotypes over two locations of Nepal

| Genotypes          | Days to heading | Days to maturity | Plant height (cm) | Panicle length (cm) | Grain yield (t ha⁻¹) | 1000-grain weight (g) |
|--------------------|-----------------|------------------|-------------------|---------------------|----------------------|-----------------------|
| Aule Kaguno        | 99.00 ± 1.51    | 135.17 ± 2.01d   | 206.80 ± 20.92abc | 21.43 ± 1.68ab      | 2.96 ± 0.03b         | 2.28 ± 0.13d          |
| CO1896(Jumla)      | 105.17 ± 7.97   | 151.33 ± 4.29a   | 206.40 ± 13.32abc | 20.03 ± 0.61bc      | 2.79 ± 0.03bcdc      | 2.55 ± 0.17bc         |
| CO5644(Lamjung)    | 98.33 ± 5.04    | 126.50 ± 5.16e   | 187.90 ± 21.73d   | 18.33 ± 0.53c       | 2.46 ± 0.03de        | 1.90 ± 0.08e          |
| CO5647(Lamjung)    | 95.67 ± 6.04    | 128.83 ± 4.42e   | 198.80 ± 16.52cd  | 18.70 ± 0.88c       | 2.44 ± 0.03de        | 1.96 ± 0.09e          |
| Kalo Kaguno        | 99.00 ± 4.27    | 150.50 ± 3.80a   | 220.23 ± 18.45a   | 23.43 ± 1.11a       | 3.58 ± 0.02a         | 2.64 ± 0.16b          |
| Pahelo Kaguno      | 98.17 ± 4.92    | 147.33 ± 2.32b   | 218.03 ± 18.91ab  | 21.97 ± 1.45ab      | 2.56 ± 0.01cde       | 2.60 ± 0.15b          |
| Rato Kaguno        | 93.33 ± 4.29    | 140.17 ± 5.00c   | 205.23 ± 22.66bc  | 23.60 ± 2.91a       | 2.25 ± 0.01e         | 2.80 ± 0.09a          |
| Seto Kaguno        | 90.83 ± 6.50    | 138.00 ± 0.68cd  | 212.73 ± 24.88abc | 21.47 ± 1.99ab      | 3.19 ± 0.02ab        | 2.45 ± 0.04c          |
| Mean               | 97.44           | 139.73           | 207.02            | 21.12               | 1.33                 | 2.40                  |

Mean values in columns with different letters are significantly different (p ≤ 0.05) according to Fisher’s LSD test.
Kaguno while CO5644 and CO5647 were earliest to 80% maturity as depicted by pooled analysis of variance. Kalo Kaguno was found to be superior as it recorded higher pooled mean for panicle length (23.43 ± 1.11 cm), plant height (220.23 ± 18.45 cm), and grain yield (3.58 ± 0.02 t ha\(^{-1}\)). Similarly, Rato Kaguno recorded the highest pooled mean value for 1000-grain weight (2.80 ± 0.09 g) followed by Kalo Kaguno and Pahelo Kaguno.

Understanding of the interaction of the traits among themselves and with the economic yield is of great use in plant breeding. Correlation studies provide information on the nature and extent of association between any two traits and it would be possible to bring out genetic improvement in one trait that could be efficiently utilized in selection of the other trait of a pair also. The Pearson’s correlation coefficients among different agro-morphological traits of foxtail millet over the two locations are presented in Table 10. Plant height exhibited highly significant positive correlation with panicle length \((r = 0.694)\), grain yield \((r = 0.512)\), and 1000-grain weight \((r = 0.411)\). Panicle length was strongly correlated to 1000-grain weight in positive direction. Similarly, grain yield showed highly significant negative correlation with days to 50% heading \((r = -0.433)\) and significant positive correlation with 1000-grain weight \((r = 0.319)\). 1000-grain weight showed significant negative correlation with days to 50% heading \((r = -0.367)\). Nirmalakumari and Vetriventhan (2010) also reported that plant height showed positive significant correlation with panicle length.

PCA measures the important traits which have a greater impact on the total variables and each coefficient of proper vectors indicated the degree of contribution of every original variable with which each principal component is associated (Sanni, Fawole, Guei, Ojo, & Somado, 2008). According to Clifford and Stephenson (1975) and Guei, Sanni, Abamu, and Fawole (2005), first three principal components are often the most important in reflecting the variation patterns among the different genotypes and the characters associated with these are most important in differentiating various genotypes. The criterion of Raji (2002) was chosen to determine the cutoff limit for the coefficients of the proper vectors. According to the devised criterion, coefficients greater than 0.3 (regardless the direction positive or negative) as having a large enough effect are considered important, while traits having a coefficient less than 0.3 are considered not to have important effects on the overall variation observed in the present study. Table 11 presents the principal component and percentage of contribution of each component to the total variation in the foxtail millet genotypes. The first principal component accounted for 60.20% of the total variation in the population. Plant height, 1000-grain weight, panicle length, days to maturity, and grain yield were the traits that contributed substantially to the first component. Second principal component accounted for 21.3% of the total variation. Days to 50% heading and days to 80% maturity contributed more in negative direction while panicle length contributed more in positive direction to the second component. The component loading correction plot of the first and the second components using the agro-morphological variables indicated plant height, panicle

### Table 10. Correlation matrix between different agro-morphological traits of test foxtail millet genotypes

|                         | Days to heading | Days to maturity | Plant height  | Panicle length | Grain yield  |
|-------------------------|-----------------|------------------|---------------|----------------|--------------|
| Days to maturity         | 0.420**         | -                 |               |                |              |
| Plant height (cm)        | -0.684**        | -0.08            | 0.694**       |                |              |
| Panicle length (cm)      | -0.374**        | 0.323*           | 0.694**       | 0.265          |              |
| Grain yield (t ha\(^{-1}\)) | -0.433**   | -0.086           | 0.512**       | 0.265          |              |
| 1000-grain weight (g)    | -0.367*         | 0.158            | 0.411**       | 0.431**        | 0.319*       |

** Correlation coefficient is significant at \(p \leq 0.01\) (2-tailed).
* Correlation coefficient is significant at \(p \leq 0.05\) (2-tailed).
length, 1000-grain weight, days to maturity, and grain yield score loaded more on the first principal component (Figure 2). The first two principal components with Eigen value $\geq 1$ accounted for 81.50% of the total variation. The present finding indicated that indirect selection based on traits such as plant height, panicle length, 1000-grain weight, and days to maturity might be assuring for identifying superior genotypes of foxtail millet. Sapkota, Pandey, and Thapa (2016) reported the traits viz., flag leaf length, flag leaf breadth, peduncle length, number of panicle per square meter were the major factors contributing to variation of foxtail millet accessions. High level of variability existing within the genotypes and the traits will make room for further improvement of the cultivars in the breeding programs.

4. Conclusion
Our studies revealed marked diversity in the studied 27 Nepalese foxtail millet landraces for various agro-morphological traits that could be effectively exploited in crop improvement program for various traits. The foxtail millet landraces were grouped into six distinct clusters independent of the geographic source of collection. The present study complements the robust breeding program.
of high yielding and climate-resilient varieties of farmer's preference by identifying and sourcing promising high yielding genotypes viz., Kalo Kaguno, Seto Kaguno, Aule Kaguno, and CO1896 and early maturing genotypes viz., CO5644, and CO5647 for diversity deployment in study sites and similar mountainous regions and provides worthy choice of genitors in breeding programs. Our studies revealed plant height, panicle length, 1000-grain weight, and days to maturity to be the principal discriminatory agro-morphological traits contributing to the total variation and substantiated that indirect selection of foxtail millet genitors based on these traits could be worthwhile for crop improvement. Similarly, the results of association study between the various traits further signified that traits viz., plant height, panicle length, 1000-grain weight, and days to maturity could be taken into consideration in breeding program for yield improvement of foxtail millet. Our findings encourage 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 region and also thrust on rapid release and registration of promising ones in state of no any variety released in Nepal to the date. We conclude diversity sourcing of neglected and under-utilized crops such as foxtail millet through diversity assessment and on-farm evaluation as an avenue to meet farmer’s need and improve food security in high altitude regions of Nepal or elsewhere. Additionally, we also thrust on the dire need to continue sourcing new diversity to best fit the functional, consumption, and location-specific requirement of the end users.

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Competing Interest
The authors declare that they have no competing interests

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