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Assessment of Variability of Foxtail Millet [*Setaria italica* (L.) Beauv.] Genotypes

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Abstract

Analysis of genetic diversity and variability is an initial step for crop development in a plant breeding program. Thirty foxtail millet genotypes were evaluated for their ten agro-morphological traits *viz.* grain yield, days to heading, days to maturity, plant height, leaf length, leaf width, number of nodes per tiller, panicle length, thousand-grain weight, and leaf blast during April to August season of 2018 at Kabre, Dolakha, Nepal. Clustering was done based on variability observed among the genotypes for all the ten traits by the average linkage method. The genotypes were grouped into six clusters. The shortest inter cluster distance between Cluster I and Cluster II was 17.63 and the longest was between Cluster IV and Cluster VI with 47.55. Cluster I had the maximum leaf blast score with the minimum leaf length. The Cluster II had the maximum grain yield and days to 50% heading and the number of nodes per tiller. The Cluster III was characterized by the maximum leaf length and leaf blast value. The genotypes grouped into the Cluster IV were characterized by the lowest value of days to 50% heading and 80% days to maturity and those of Cluster V had the maximum thousand-grain weight. The genotypes categorized into Cluster VI had the maximum plant height, leaf length, and panicle length. Cluster II and Cluster V were good because of their agro-morphological traits. The presence of a high level of diversity among the genotypes indicated their suitability for selection in crop breeding programs. Correlation analysis of genotypes having the maximum panicle length leaf area and higher plant height and shorter maturity period are pre-requisite for improvement in grain yield.

Keywords: Cluster analysis, diversity, Foxtail millet, grain yield

Introduction

Foxtail millet (*Setaria italic* (L.) Beauv.) is a self-pollinating crop with chromosome numbers, $2n = 18$, classified under the Poaceae family and subfamily Panicoideae (Fedorov, 1974). It is one of the cultivated cereals and extensively cultivated in India, Nepal, Sri Lanka, Pakistan, Russia, Ukraine, the Middle East, Turkey, and Romania (Bala, 2004). It is hardy in nature and generally grows well on marginal lands having scarcity of irrigation as rain-fed crops (Dai *et al.*, 2011a; 2011b). Its cultiva-

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tion was domesticated around 8,000 years ago in the highlands of central China (Lu *et al.*, 2009; Amgai *et al.*, 2011). Now it is cultivated in 26 countries (Baker, 2003). Foxtail millet ranks second among the millets as for world production (Marathee, 1993; Bala, 2004). Millet is an important cereal crop in Nepal, cultivated on 271,183 ha with the production of 304,105 mt and productivity of 1,121 kg/ha (MoAD, 2016). It is also a good source of energy, fats, proteins, fatty acids, vitamins, minerals and dietary fiber (Jali *et al.*, 2012).

The correlation studies measure the associations between yield and other traits. It helps to determine the association of traits with grain yield through correlation coefficient analysis. Identification and release of a promising variety of foxtail millet is the most promising and deliverable technology for increasing productivity through its utilization in crop improvement programs. Kandel *et al.* (2019) evaluated the performance of finger millet landraces over different environments, which is necessary for plant breeders in selection and utilization in crop breeding programs.

Genetic diversity analysis is imperative in crop improvement and can be studied through morphological, biochemical, and molecular markers. Morphological characterization for genetic divergence among genotypes is considered an initial step (Khan *et al.*, 2014). Therefore, morphological data has to play a key role in the management of genetic resources. For the management of genetic resource; study of relationships, description and classification of germplasm, and morphological characterization are the first step (Smith and Smith, 1989). In plant breeding, cluster analysis techniques help to separate the varieties into homogeneous groups from the heterogeneous groups such that those varieties within a group have similar traits to each other in response patterns across the locations. Genetic diversity study on accessions of foxtail millet can provide pivotal information for the breeding of crops and in the management of genetic resources. Evaluation of local foxtail millet landraces is necessary for the utilization in crop improvement. Also, the cluster study of these landraces can be pivotal to identify accessions with diverse traits, which can be helpful in breeding programs. The diversity study of foxtail millets will help to explore the genetic variability available in Nepal, which ultimately contributes to the exploitation of genetic resources for future breeding research. The objective of the present study was to characterize the level of variability in foxtail millet accession collected from different parts of Nepal and to analyze genetic diversity and association among economically important traits for utilization in the breeding programs.

Materials and Methods

Experimental site and planting materials

The experiment was conducted in Kabre of Dolakha district from April to August 2018. The research field in Kabre was located in the mid-hill region and thus was characterized by cool temperate climate. The geographic coordinates for the research field were $86^0$9' E longitude, $27^0$38' N latitude, and 1740 m altitude. The soil at the research field at Kabre was sandy loam with pH ranging in between 4.5 to 6.2, i.e., slightly acidic (NARC, 2018). The climatic data for the experiment period, between April to August in 2018, are given in Table 1.

Experimental design, field layout, and cultural practices

A total of 30 Foxtail millet germplasms were maintained and evaluated. The trial was conducted using rod row design. Fertilizer application was done at the rate of 50:30:20 kg of N:P:K per ha (HCRP, 2019). Half a dose of N and full doses of P and K were applied as basal doses and the remaining half of N was applied as side-dressing at the time of the tillering growth stage. The plots were kept free of manual weeding. The seeds

Table 1: Climatic data from April to August 2018 in Kabre, Dolakha in 2018

Months	Max. temp. (^{0}C)	Min. temp. (^{0}C)	Total rainfall (mm)
April	26.5	12.5	73.6
May	28.5	13.3	180.3
June	28.8	17	181.3
July	28	18.5	552.6
August	27.5	19	378.4

(Source: HCRP, 2019)

were sown 25 cm row to row distance and 5-10 cm plant to plant distance in six rows of 2 meter length**.** The panicles in each plot were harvested separately by cutting from the peduncle base and placed in paper envelops.

Research treatments

A total of 30 prosomillet germplasms were maintained and evaluated. The foxtail millet genotypes were received from Hill Crops Research Program, Dolakha, Nepal for these experiments. The source of these foxtail millet genotypes was Nepal Agricultural Research Council, Hill Crops Research Program, Kabre, Dolakha, Nepal. All genotypes were of lines and their origin was in Nepal.

Data collection

Data on grain yield and yield attributing traits were recorded according to the foxtail millet descriptor (IBPGR, 1985). Each plot of 3 m^2 was harvested excluding border rows and grain moisture content for each plot was recorded, and grain yield was adjusted to a 12% moisture basis. The leaf blast scoring (1-5) was done using a protocol (HCRP, 2018**).** The leaf width was calculated using five sample plants and data were measured from three places at the base, mid, and tip and averaged. Leaf length was measured for the length of five leaves (base to apex). Flag leaf angle was characterized as erect, intermediate, horizontal, and descending. Node color was classified and measured as red, white, green, light green, and purple during the physiological maturity of the crop. The grain yield per plot was converted into ton/ha by using the formula given below (HCRP, 2018).

$$\text{Grain yield}\left(\frac{t}{ha}\right) = \frac{\text{Yield of plot (kg)} \times 10 \times (100 - \text{HMP})}{\text{NPA} \times (100 - \text{DMP})}$$

Where,

HMP = Grain moisture percentage at harvest

DMP = Desired moisture percentage, i.e. 12%

NPA = Net harvest plot area, m^2

Statistical analyses

The data recorded on different parameters from field were first tabulated and processed in Microsoft Excel (MS-Excel, 2010). Correlation coefficients of different traits using SPSS program were analyzed using the Steel and Terrie (1980) formula. The genetic diversity was observed in agro-morphological traits namely the grain yield, days to heading, days to maturity, plant height, leaf length, leaf width, number of nodes per tiller, panicle length, thousand grain weight, and leaf blast using Minitab 18.

Results and Discussion

Agromorphological evaluation

Five genotypes produced grain yield more than 1 t/ha (Table 2). These were HUMLA-252 (1.86 t/ha), CO1896 (1.15 t/ha), HUMLA-606 (1.12 t/ha) and CO3474 (1.05 t/ha). The promising genotypes were HUMLA-522 (0.693 t/ha), HUMLA-523 (0.423 t/ha) and HUMLA-163 (0.86 t/ha). The average leaf blast value (1-5) of Blast disease was 2.1. These high yielding genotypes possessed leaf blast value of 1.7.

Table 2: Agro-morphological traits of 30 foxtail millet genotypes at Kabre, Dolakha in 2018

SN	Name	DTH	DTM	PH	LL	LW	NNT	PL	TGW	GY	LB (1-5)
1	CO1896	64	109	116	17	1	8	12	1.92	1.153	1.75
2	CO3474	83	114	120	25	1	8	12	1.8	1.053	1.75
3	CO3475	52	98	130	26	1	7	13	1.92	0.247	1.75
4	CO4576	68	105	109	16	1	6	9	2.4	0.207	1.75
5	CO4577	48	80	114	16	1	6	8	1.6	0.087	3.15
6	CO4579	62	106	122	19	1	9	13	1.76	0.06	3.15
7	CO4580	57	97	112	25	1	7	13	1.24	0.933	2.8
8	CO5148	61	103	108	14	1	6	9	2.02	0.313	2.1
9	HUMLA-21	68	105	127	18	1	8	7	2.18	0.353	2.1
10	HUMLA-76	56	80	125	23	1	7	7	2.4	0.24	1.75
11	HUMLA -77	52	79	101	24	1	7	11	1.98	0.253	1.75
12	HUMLA -85	53	81	120	26	1	7	15	2.24	0.213	1.75
13	HUMLA -149	68	102	118	19	1	8	10	2.12	0.333	1.75
14	HUMLA-150	74	102	121	19	1	7	11	1.9	0.4	1.75
15	HUMLA-163	70	105	129	18	1	9	13	1.42	0.86	1.75
16	HUMLA-164	73	108	118	24	1	8	12	2.2	0.567	1.75
17	HUMLA-213	72	106	110	18	1	6	7	1.94	0.667	1.75
18	HUMLA-251	72	114	109	13	1	8	7	2.46	0.233	1.75
19	HUMLA-252	69	115	137	14	1	7	11	2.86	1.867	1.75
20	HUMLA-314	75	116	98	21	1	7	8	2.52	0.247	1.75
21	HUMLA-378	68	111	116	13	1	6	7	2.22	0.407	1.75
22	HUMLA-379	69	111	128	14	1	8	9	2.34	0.327	1.75
23	HUMLA-380	67	107	119	15	1	8	9	2.04	0.213	1.75
24	HUMLA-468	70	113	110	18	1	6	10	2.76	0.733	1.75
25	HUMLA-469	58	100	133	25	2	8	16	2.52	0.127	1.75
26	HUMLA-522	57	74	113	20	1	8	14	2.2	0.693	1.75
27	HUMLA-523	70	107	114	19	1	8	11	2.18	0.467	3.85
28	HUMLA-524	68	119	130	21	2	7	13	2.48	0.567	3.85
29	HUMLA-606	57	119	134	25	1	7	14	2.6	1.127	1.75
30	HUMLA-631	55	122	139	28	1	8	15	2.32	0.907	1.75
	Mean	65	104	119	20	1	7	11	2	1	2.1
	SE Mean	1.53	2.35	1.88	0.809	0.05	0.161	0.495	0.067	0.074	0.115
	CV (%)	12.98	12.42	8.64	22.42	23.8	12.06	24.97	17.21	16.96	10.89

DTH=50% days to heading, DTM=80% days to maturity, PH=plant height, LL=leaf length, LW=leaf width, NNT=Number of node per tiller, PL=panicle length, TGW=thousand grain weight, GY=grain yield (t/ha), LB=leaf blast

Table 3: Agro-morphological traits of 30 foxtail millet genotypes within and among six clusters

Variables	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Grand centroid
Days to heading (50%)	68.5	83	55.667	53.2	75	56	64.533
Days to maturity (80%)	108.222	114	98.333	78.8	116	120.5	103.6
Plant height	118.944	120	125	114.6	98	136.5	119.333
Leaf length	17.167	25	25.333	21.8	21	26.5	19.767
Leaf width	1.056	1	1.333	1	1	1	1.067
Number of node per tiller	7.389	8	7.333	7	7	7.5	7.333
Panicle length	10	12	14	11	8	14.5	10.867
Thousand Grain weight,	2.178	1.8	1.893	2.084	2.52	2.46	2.151
Grain yield	0.54	1.053	0.436	0.297	0.247	1.017	0.528
Leaf blast (0-9)	2.1	1.75	2.1	2.03	1.75	1.75	2.042

The mean value of 50% days to heading, 80% days to maturity, plant height, leaf length, leaf width, number of nodes per tiller, panicle length, thousand-grain weight, grain yield, and leaf blast were 65, 104, 119, 20, 1, 7,11, 2, 1.01 and 2.1 respectively. Among the tested genotypes, 50% days to heading was the highest with 83 days in C03475 and the lowest of 52 days in C03474 and HUMLA-77. Similarly, days to 80% maturity was highest with 122 days in HUMLA-631 and lowest with 74 days in HUMLA-522 and HUMLA-77. Plant height ranged from 139 cm (HUMLA-631) to 98 cm (HUMLA-314). Leaf length varied from 28 cm (HUMLA-631) to 13 cm HUMLA-378 and HUMLA-251. Number of nodes on tiller varied from 9 (HUMLA-163, CO4580) to 6 (HUMLA-378, HUMLA-468, HUMLA-213 and C05180). Thousand-grain weight varied from 2.85 g in HUMLA-252 to 1.6 g in C04575. The grain yield variation was highest (1.86 t/ha) in HUMLA-251 and lowest (0.4 t/ha) in HUMLA-150.

The observed variation in panicle length may be attributed to differences at the genotypic level. Panicle length was highest (16 cm) in HUMLA-496 and lowest (7 cm) in HUMLA-251, HUMLA-213, HUMLA-378, HUMLA-21 and HUMLA-76. Kamatar *et al*. (2014) also found similar findings of the presence of significant variability in foxtail millet genotypes for grain yield and yield attributing traits. In this study, considerable morphological variation was found mainly due to genetic factors and also subjected to environmental factors (Table 3, Figure 1). The findings of our study was similar to the findings reported by Reddy *et al*. (2006) who conducted a similar study on 1,535 foxtail millet accession from 26 countries that included 21 accessions, and the millet from Nepal had higher grain yield. A similar finding of the presence of significant phenotypic diversity in the Chinese foxtail millet genotype was reported by Li *et al*. (1996). Babu (2019) and Gangurdei *et al*. (2016) also reported a similar type of significant variation on genetic diversity on foxtail millet genotypes. The present study also suggested that high yielding accessions of foxtail millet may be selected by indirect selection of plant height, panicle length, leaf width, and length. In a previous study by Reddy *et al*. (2006) in which they had collected and characterized 1,535 foxtail millet accessions from 26 countries that included 21 accessions from Nepal had a higher grain yield than in the present study.

Clustering pattern of genotypes under this study indicated that the foxtail millet genotypes show considerable genetic diversity among themselves by occupying six different clusters (Table 3). These genotypes were grouped mainly based on observed traits data such as 50% days to heading, 80% days to maturity, plant height, leaf length, leaf width, number of nodes per tiller, panicle length, thousand-grain weight, and leaf blast. The values falling within the clusters are presented in Table 3. Based on a variation of the observed agro-morphological traits among the 30 genotypes, a cluster analysis was conducted. Cluster I comprised of 18 genotypes, Cluster II of 1, Cluster III of 4, Cluster IV of 5, Cluster V of 1 and Cluster VI of 2 genotypes (Figure 1 and Table 5). The genotypes grouped into Cluster I had the maximum leaf blast score with the minimum leaf length. The genotypes grouped into Clusters II had the maximum grain yield and days to 50% heading and the number of nodes per tiller (Table 3). Cluster III was characterized by the maximum leaf length and leaf blast value. The genotypes grouped into the Cluster IV were characterized by the lowest value of days to 50% heading and 80% days to maturity and those of Cluster V had the maximum thousand-grain weight. The genotypes categorized into Cluster VI had the maximum plant height, leaf length, panicle length, and comparatively early heading whereas the same traits grouped into Cluster II had late heading time. The distance between clusters was calculated based on recorded 10 agro morphological traits among 30 genotypes of foxtail millet by using Minitab software. It helps to know the diversity among genotypes. Based on cluster distance analysis, genotypes belonging to Cluster I, II, III, V and VI were more or less similar in performance whereas Cluster V had a significant difference (Table 4). The estimation of diversity and relationships among germplasm accessions facilitates the selection of parents with the diverse genetic background which is very essential for the breeding program (Nakayama *et al*., 1999; Brunda *et al*., 2014; Kandel *et al*., 2017). The lower grain yields in this study might be

Table 4: Distances between cluster centroids of 30 foxtail millet genotypes within and among six clusters

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	0					
Cluster II	17.6355	0				
Cluster III	19.5454	31.9802	0			
Cluster IV	33.7835	46.5748	22.75	0		
Cluster V	23.6767	24.2109	38.3463	46.3093	0	
Cluster VI	26.8886	32.4451	25.0251	47.557	44.0096	0

Table 5: Grouping of 30 foxtail millet genotypes into six clusters on agro-morphological traits

Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
C01896, HUMLA380, HUMLA-378, C04579, HUMLA149, HUMLA -150, HUMLA-164, C04576, HUMLA-213, HUMLA-523, HUMLA-251, HUMLA-468, C05148, HUMLA-21, HUMLA-163, HUMLA-379, HUMLA-252, HUMLA-524	C03474	C03475, HUMLA-469, C04580	C04577, HUMLA-522, HUMLA-76, HUMLA-85, HUMLA-77	HUMLA-314	HUMLA-606, HUMLA-631

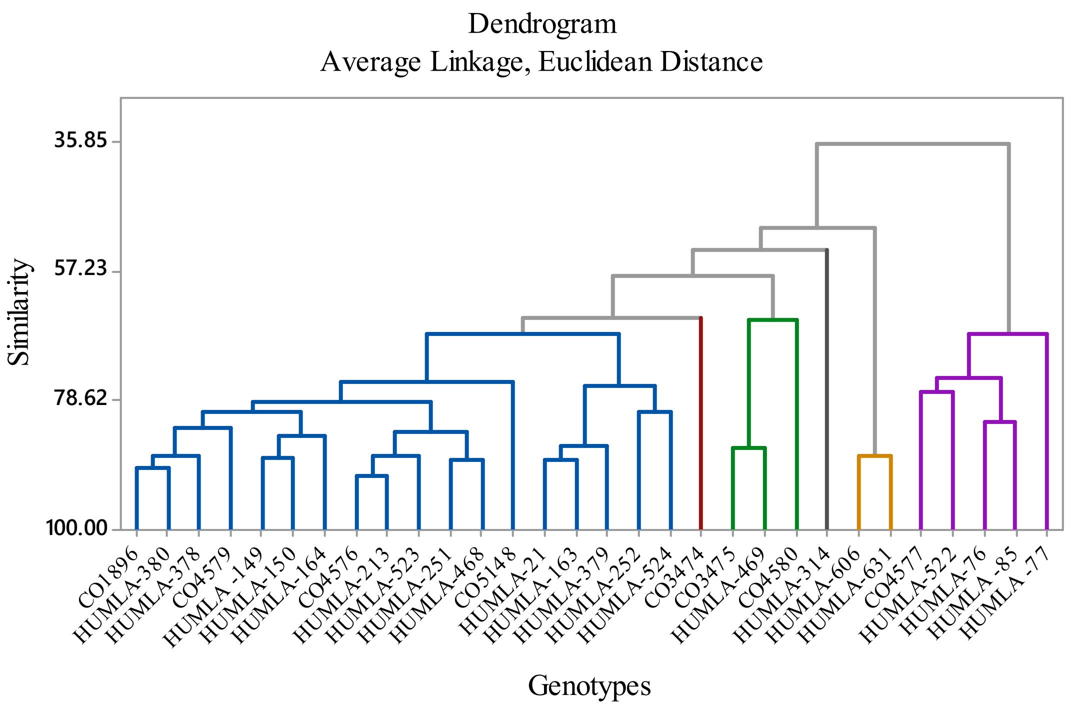

Figure 1: Cluster analysis of 30 foxtail millet genotypes for agro-morphological traits in 2018 HCRP, Dolakha

due to the infestation of disease and pests.

Groupings based on principle component analysis

Principle component analysis (PCA) helps to partition the total variance due to recorded traits among the tested genotypes into different components which help to study diversity. The PCA analysis and distinct clusters with important phenotypic attributes contribute towards diversity and provide a base for the selection of desirable genotypes with specific traits for breeding programs. PCA showed 4 principle components or factors (PC1, PC2, PC3 and PC4) having Eigenvalue >1 explaining 73.1% of the total variation (Table 6). It means that the 73.1% variation is due to agro-morphological traits and remaining 26.9% is due to unknown factors. The first principle component (PC1), which explained 24.4% variation, was associated mainly with panicle length, leaf length, plant height, and leaf width. The second principal component (PC2) explains 21.7% of the variation and was mainly related to days to maturity, days to heading, thousand-grain weight, leaf blast, and grain yield which shows a similar result with Cluster II in cluster analysis (Figure 2). The third principle component (PC3) explains about 13.5% of the variation and was mainly related to leaf width, leaf blast value and grain yield. The fourth principle component (PC4) explains 12.6% of the variation and was mainly related to thousand-grain weight, number of nodes per tiller, and leaf blast value. Thus, principle component analysis supports the result of cluster analysis among the tested genotypes.

Phenotypic correlation among foxtail millet genotypes

The phenotypic correlation analysis showed that grain yield had significant positive correlation with plant height ($r = 0.353$, $p < .05$) and leaf length ($r = 0.058$, $p < .05$). Similarly, grain yield had significant negative correlation with days to heading ($r = -0.214$, $p < .05$) and days to maturity ($r = -0.389$, $p < .05$). The genotypes with a high value of plant height and panicle

Table 6: Principle component analysis for ten agro-morphological traits in 30 foxtail millet genotypes

Variables	PC1	PC2	PC3	PC4
Eigen value	2.536	2.168	1.349	1.256
Proportion	0.254	0.217	0.135	0.126
Cumulative	0.254	0.47	0.605	0.731
Days to heading (50%)	-0.225	-0.498	-0.006	0.306
Days to maturity (80%)	0.022	-0.592	-0.116	0.116
Plant height	0.449	-0.237	0.009	-0.074
Leaf length	0.45	0.194	0.155	-0.152
Leaf width	0.31	-0.062	-0.644	-0.08
Number of node per tiller	0.295	-0.123	0.195	0.539
Panicle length	0.567	0.053	0.097	0.006
Thousand Grain weight	-0.004	-0.346	-0.263	-0.597
Grain yield	0.176	-0.387	0.362	-0.072
Leaf blast (1-5)	0.091	0.131	-0.548	0.454

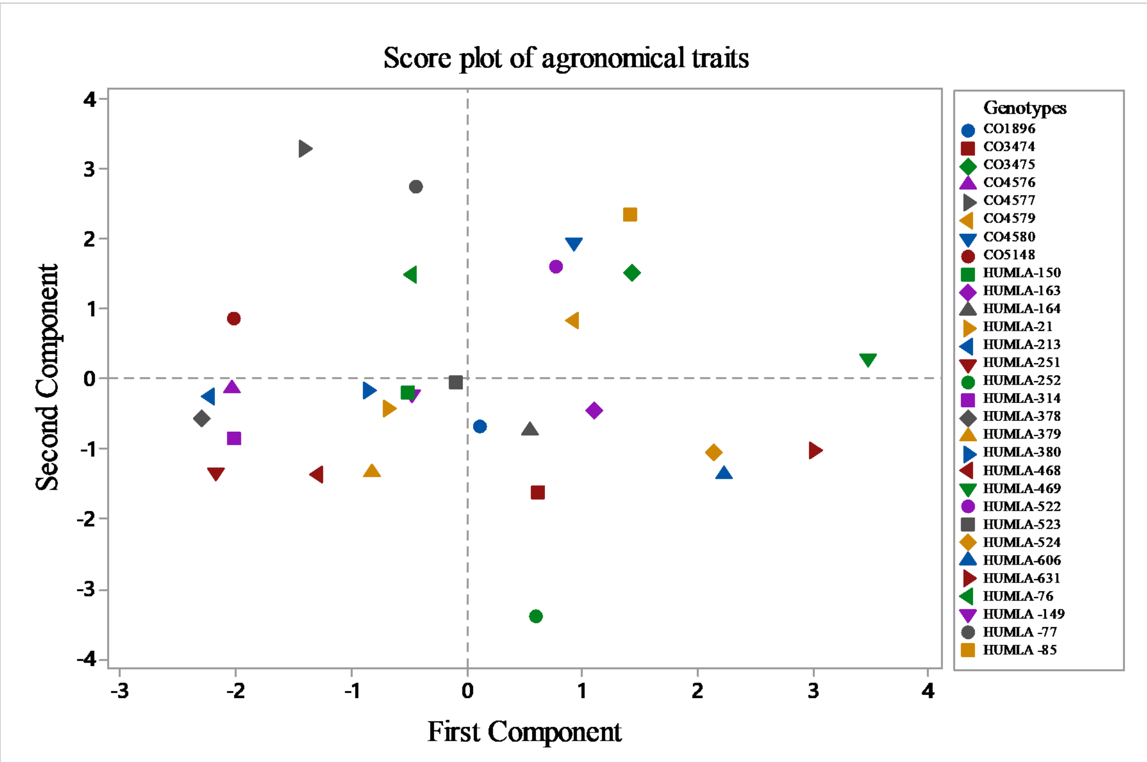

Figure 2: The score plot of first two components of foxtail millet genotypes at HCRP, Dolakha (2018)

length and shorter heading and flowering days produced the maximum grain yield under the study conditions. Khaliq *et al.* (2008) reported similar result in which grain yield is positively correlated with plant height and panicle length. However, Amgai *et al.* (2011) reported that grain yield is negatively correlated with days to heading and maturity, but reported significant positive correlation between plant height and panicle length on five Nepalese foxtail millet accessions. The number of days between the maturity time and heading time de-

Table 7: Pearson's correlation coefficient among different traits of foxtail millet genotypes

	DTH	DTM	PH	LL	LW	NNT	PL	TGW	GY
DTM	0.615**	1							
PH	-0.149	0.274	1						
LL	-0.363*	-0.168	0.261	1					
LW	-0.05	0.125	0.321*	0.198	1				
NNT	0.166	0.124	0.362*	0.161	0.051	1			
PL	-0.335	-0.032	0.468**	0.669**	0.364*	0.393*	1		
TGW	0.148	0.333*	0.181	-0.09	0.256	-0.191	-0.08	1	
GY	-0.214*	-0.389*	0.353*	0.058*	0.121	* 0.054	0.282	* 0.120	1
LB	-0.104	0.013	-0.008	-0.031	0.327*	0.036	0.08	-0.231*	-0.139*

DTH=50% days to heading, DTM=80% Days to maturity, PH=Plant height, LL=leaf length, LW=Leaf width, NNT=Number of node per tiller, PL=Panicle length, TGW=Thousand Grain weight, LB =Leaf blast (1-5), **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

notes the grain-filling period. The longer grain filing period is a desirable combination that the breeders are interested to find. In this study, the correlation analyses indicated that those accessions which mature early i.e., shorter grain filling duration, yielded higher (0.297t/ha Table 3) compared to the late maturing accessions (0.247t/ha Table 3). The accessions that matured late might have experienced heat stress during grain filling and therefore suffered from yield loss. Heat stress is considered as one of the major environmental factors decreasing the crop yield as the stress induces many molecular, biochemical, and physiological changes, which affect the crop growth and the grain yield negatively (Prasad *et al.*, 2008).

In this study days to heading and days to maturity were negatively correlated with the grain yield indicating that early maturing materials had higher grain yields. These findings are similar to the findings reported by Amgai *et al.* (2011). Successful breeding of high yielding varieties depends on the yield contributing morphological traits and choosing a small number of important traits having a positive correlation. According to Khaliq *et al.* (2008), the agronomic traits such as the plant height, leaf length, leaf width, and panicle length are positively correlated with grain yield and negatively correlated with days to heading and maturity and leaf blast.

Conclusion

A diversity in agro-morphological traits namely grain yield and days to heading, days to maturity, plant height, leaf length, leaf width, number of node per tiller, panicle length, thousand grain weight, and leaf blast among 30 foxtail millet genotypes, which indicate scope for selection of desirable genotypes with specific traits for breeding program was observed in a field experiment in Nepal. The genotypes grouped into Cluster II and Cluster V are good because of their yield attributing agro-morphological traits such as shorter flowering period, maturity days and lower leaf blast score and the maximum value for thousand grain yield, leaf length, panicle length and grain yield. The correlation analysis showed that genotypes having the maximum panicle length, leaf area, higher plant height and shorter maturity period are prerequisite for enhancing grain yield. Thus, the presence of a high diversity among the genotypes grouped into different clusters indicates their suitability for selection in crop breeding program.

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