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Evaluating exotic foxtail millet accessions for growth, vigor, and yield adaptation under Faisalabad climate

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Millets are becoming popular due to special features of high nutrition, low inputs, and climate resiliency. While major crops like wheat, rice & maize are exhaustive, resource-loving, and susceptible. There is a need for crop diversification & potential millets to be adapted for sustainability. Faisalabad is the grain belt of Punjab, Pakistan, but only one millet crop, pearl millet, is being cultivated for grain purposes. An alternate crop, foxtail millet study was designed, and 200 exotic accessions of different origins were acquired from the USDA. Germination behavior at optimal controlled temperatures (18 °C, 25°C, 32°C, and 39 °C) was tested, best germination was observed at 32 °C, and 135 out of 200 accessions germinated at ≥80%. These accessions were initially screened for germination strength (G%, G50, MGT, U, GI, Z, and CVG), seedling vigor (SL, RL, SFW, SDW), and preliminary yield tests in the greenhouse, then evaluated under three different field locations of varied soil environments. Principle component analysis (PCA) was performed, and 14 clusters of accessions were sorted out using hierarchical clustering. The high-yielding accessions were selected from each cluster and sown at three different locations/soil environments in the Faisalabad, i.e., 1- ARA, UAF (fertile, low irrigated), 2= DFP, UAF (salt-affected soil), 3= JKA, CJF (fertile, well-irrigated). Results were statistically analyzed & graphically represented using project R for statistical computing. Accessions A73-IN and A101-IN produced the highest grain yield of 3504 kg ha^{-1} and 2667 kg ha^{-1} respectively, and a biomass accumulation of 8 t ha^{-1} at the location JKA, CJF. Yield-related results under low irrigation & saline soil were also significant, however, overall, 69% of yield was reduced in the saline and 67% in low irrigated fields. It was concluded that accessions of Indian origin may be adapted under Faisalabad climate and an additional inquiry into abiotic stresses is required to discover suitable genotypes.
Keywords: Foxtail millet, Faisalabad, High nutrition, Setaria, Viridis, Italica

INTRODUCTION

Millets are small rounded grains available in many colors and forms of the Poaceae family, including Maize and Sorghum (Sharma and Niranjan, 2018). Foxtail millet is an economically important (Sheahan, 2014) and widely cultivated food crop (Austins, 2006; Ahmad *et al.*, 2018) in the arid and semi-arid regions of Asia and Africa, with a long history of cultivation. However, fifty-five years ago, foxtail millet was unknown to maize fields in North America (Dekker, 2003). Then, quickly introduced and cultivated, indicating that this species can be embraced in broader climates (Dekker, 2003).

Millets are the powerhouse of nutrients and have a great potential for the sustainability of food, nutritional security, and human health. (Nithyashree *et al*., 2020). A new range of millet products has been introduced in the market including drinks, instant millet powder, bread cakes, etc. (Saleh *et al*., 2013). Foxtail millet has been included in healthy diets due to its easy digestibility, low glycemic contents, gluten-free protein, and high iron content (Yin *et al*., 2019). Foxtail millet is generally used as a porridge or cooked grain in the same way as rice and may be mixed with any other grain (He *et al*., 2015). Foxtail millet is rich in vitamins, amino acids, and minerals. Foxtail millet has two to three times higher nutritional contents than the widely flourished major crops wheat and rice (M. Y. Kamatar *et al*., 2015). It has 12.3 g protein, 60.9 g carbohydrates, 4.3 g fat, 8 g crude fiber, 3.3 g mineral matter, and 351 g per 100 g of matter with 351 Kcal of energy (Sharma and Niranjan, 2018).

Foxtail millet has tremendous genetic diversity, and its genome is being preserved in various divisions of genetic capital in China, Japan, India, and the USA (Moharil *et al.*, 2019). The genome sequence of foxtail millet has been

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unlocked (Li and Brutnell, 2011; Bennetzen *et al.,* 2012; Zhang *et al.,* 2012; Daverdin *et al.,* 2015), and its genomic features include a low number of repetitive DNA, the small genome size (~490 Mb), diploid nature (Tsai *et al.,* 2016), resemblance with other grasses like maize and sorghum (Kellogg, 1998; Devos and Gale, 2000). What makes foxtail millet unique and attractive to the scientific community is its competitive features, less plant height, photosynthetically efficient, abundant seed production, and high nutrition. Foxtail millet has superior characteristics to survive in harsh environments and stressed conditions, which makes this crop ideal for the improvement of other bioenergy grasses, and the study of wheat, maize, barley, and oat (Jia *et al.,* 2015; Jia, 2017). Foxtail millet and its wild progenitor green foxtail are extensively studied and are now considered a model crop for studying C4 photosynthesis, stress biology, and bioenergy-related traits (Muthamilarasan and Prasad, 2015). This information is beneficial for studying the stress biology of crops and ensures ecological security (Hariprasanna, 2016). The world food challenge (feeding the steadily increasing world population) and agrarian challenge (threats to the major crops due to climate change) need immediate attention towards the millet crops proliferation (Bandyopadhyay *et al.,* 2017).

Agronomic features of foxtail millet include the ability to grow in harsh environments, shorter life span (90-120 days), small area of the leaf, low inputs requirements, self-pollinating behavior, excellent resistance to abiotic stresses (Zhang *et al.,* 2012; Pan *et al.,* 2020). Further, foxtail millet is a short-statured crop and variable from 20 to 215 cm depending upon accession and origin type (Reddy *et al.,* 2006), which has a short life cycle of 5 to 8 weeks from planting to flowering, and 8 to 15 weeks from planting to maturity ((Doust *et al.,* 2009). Foxtail millet is not only climate-resistant but also a nitrogen and phosphorus-nutrient-efficient crop (Nadeem *et al.,* 2020). Agronomic traits viz. grain yield/plant, number of panicles/plant, 1000-grain weight, panicle weight / main stem, and panicle weight/plant are the main targets of modern plant breeding these days for the improvement of foxtail millet (Jia *et al.,* 2015). If we see in terms of production, foxtail millet is the second most widely cultivated among millets (Singh *et al.,* 2017) and sixth-highest yielding grain in the world (Saleh *et al.,* 2013; Brink *et al.,* 2006)). Many early domesticated plants that survive themselves when there was no fertilizer, pesticides, or proper irrigation facility can be selected as naturally stress-tolerant plants NRTCs; and foxtail millet being one of them (Zhang *et al.,* 2018).

The district of Faisalabad is situated on the flat plains of northeastern Punjab, between longitude 73°74 east and latitude 30°31.5 with an elevation of 180 meters above sea level (Haq *et al.,* 2016). Although all crops are cultivated in Faisalabad, the major crops include wheat, rice, maize, sugarcane, and cotton (Agriculture Statistics, 2017), however, it is a mixed cropping zone including other minor crops like pearl millet & sorghum (Hussain *et al.,* 2011; Shahbaz *et al.,* 2017), and 80& of the total area is irrigated. All major cereal crops are nutrients, water and resource exhaustive in nature and contribute more towards global warming in comparison to alternate millet crops (Saxena *et al.,* 2018). Major crops are becoming a matter of food insecurity, also because of vulnerability to biotic & abiotic stresses (Saxena *et al.,* 2018). In this scenario, foxtail millet may be adapted in Faisalabad, Punjab, Pakistan for crop diversification and food security. Here, only pearl millet and sorghum are being grown as subsistence summer millet grain crops (Ahmad *et al.,* 2013). Further, for strengthening the millet cropping system, alternate millet crop species might be introduced, and included in the cropping system, while foxtail millet is one of the best options among them. Agricultural productivity depends upon two phenomena; continually changing climate, and genome selection according to changes in weather patterns. So, in this way, the study of exploiting genetic diversity sources of foxtail millet was performed to introduce foxtail millet into the prevailing Faisalabad cropping system.

MATERIALS AND METHODS

The experiment was designed in 2021-2022 in the climatology lab, University of Agriculture, Faisalabad. The two hundred foxtail millet accessions requested from the USDA germplasm unit were collected and sown in Petri dishes under laboratory conditions at different temperatures (18 °C, 25°C, 32°C, and 39 °C) & relative humidity (50% and 60%). This temperature range was decided based on the study of (Koocheki *et al.,* 2006; Nejad *et al.,* 2009). The germination percentage (G%) of the seed population was calculated and only the seeds with more than 80% germination were selected for further study. Foxtail millet germinable seeds (80%) were sown in Petri plates between two moist layers of filter paper. Following is the equation given by Coolbear *et al.,* 1984, which was later improved by Farooq *et al.,* 2005.

$$\mathrm{G50} = t_1 + \frac{\left(N/2 - n_1\right)(t_2 - t_1)}{n_2 - n_1}$$

Here, N is the last germination count, and t_1 and t_2 are two inline counts at time t_1 and t_2 respectively. For this equation provided is that $n_1 > N/2 > n_2$ Daily data recording of seed germination was done according to the Association of Seed Analysts (1987) (Bradnock, 1990), and data for G_{50} germination was recorded using the above-given equation. Germination rate and time spread is an important indicator that was given by (Ellis and Roberts, 1981)

$$MGT = \frac{\sum Dn}{\sum n}$$

n = No of seeds germinated on day D = No. of days from germination initiation, the Mean germination rate is the reciprocal of the mean germination time as shown below (Ranal *et al.,* 2009).

$$MGR = 1/\text{MGT}$$

The degree of uncertainty associated with relative frequency ($fi = n_i/\sum_{i=0}^{n} n_i$) was calculated as:

$$Un = \sum_{\text{i}=0}^{\text{k}} fi\ log_2\, fi$$

GI was estimated by the following equation given by the Association of Official Seed Analysts (1990).

$$GI = (No.of\ germinated\ seeds) / (\text{Days of the first count}) + + + + (No.of\ germinated\ seeds) / (\text{Days of final count})$$

Synchrony of Germination Process (Z) was calculated using the following formula

$$Z = \frac{\sum_{\text{i}=1}^{\text{k}} Cn_{\text{i},}2}{C\sum ni,2}$$

Where; $Cn_{\text{i},}2$ = ni(ni−1)/2 $Cn_{\text{i},}2$ = $n_i(n_i-1)/2$, $Cn_{\text{i},}2$ = combination of seeds germinated in the ith time, two by two. ni= number of seeds germinated in the ith time. Then the Coefficient of the velocity of germination CVG % was calculated by the expression:

$$CVG = \frac{\sum_{\text{i}=1}^{\text{k}} n_i t_i}{\sum_{\text{i}=1}^{\text{k}} n_i} \times 100$$

Germinable accessions were selected and sown in pots in the greenhouse under controlled temperature (30-35 °C) and relative humidity (50 to 60%) for the seedling vigor test. Fifteen days after emergence root length (RL) and width (RW) were measured quickly after harvesting of 5 plants as destructive sampling. For dry weight, fresh roots, and shoots were initially shade dried and then kept at 70 °C for oven drying. Root fresh weight (RFW) and root dry weight (RDW) were measured. The other plants were left for growth and development until the final yield was obtained to get the preliminary yield analysis of the accessions.

Principle component analysis was applied to the germination strength, seedling vigor, and preliminary yield from a greenhouse experiment, and the germplasm found with similarities in G%, G50, MGT, U, GI, Z, CVG and RL & SL were predicted by cluster analysis using circular hierarchical dendrogram which splits the lot of 135 accessions into fourteen different clans. One elite genotype from each accession clan with a high yield from the preliminary yield test was selected. These fourteen accessions were then evaluated under field conditions. The following three locations were selected with varying soil environments for sowing elite accessions in Faisalabad to assess the agronomic behavior.

***Location1= Area Soil Type-1*: *(Limited irrigation 2 to 3 / fertile soil)*:** Loc.1 = “Agronomic Research Area, University of Agriculture, Faisalabad / (ARA, UAF)” GPS Coordinates: 31° 26' 30.264" N, 73° 04' 27.822" E

***Location2=Area Soil Type-2*: *[Well irrigated 4 to 5 / natural saline (EC=3)]*:** Loc.2 = “Director Farms, Proka-2 University of Agriculture, Faisalabad” / (DFP, UAF)” GPS Coordinates: 31° 25' 52.3704" N, 72° 59' 8.90" E

***Location 3=Area Soil Type-3: (Well irrigated 4 to 5 / fertile conditions)*:** Loc.3 = “JK Agricultural Farms, Chak Jhumra Road, Faisalabad / (JKA, CJF)” GPS Coordinates: 31° 33' 24.2424" N, 73° 08' 30.92" *E*

Figure 1. **Sowing of 135 germinable foxtail millet accessions for seedling vigor and preliminary yield trial evaluation**

According to Prabhakar *et al.* (2017), improved production technology was studied and also plant guide for foxtail millet by Sheahan (2014) was consulted. In the initial greenhouse experiment, most of the genome was checked for the season selection. However, July August was found the best season to grow FM in the Faisalabad. Planting geometry for the experiment was kept as under:

row to row (r×r) distance = 60 cm

plant to plant (p×p) distance = 9 cm

To test the agronomic behavior of the selected accessions different yield and yield-related parameters were analyzed. Tillers per plant (TPP) were counted. The potential of the crop to produce tillers is directly related to the crop yield potential. A small number of tillers will produce less yield. Randomly 10 hills were selected from each experimental plot. Panicles per plant (PPP) were counted. According to the criterion suggested (Quinn and Wetherington, 2002; Fang *et al.*, 2004), the date on which each accession produced the first panicle emerged from the flag leaf was considered a flowering start. Mid-flowering as each accession reached half of the total panicle created. The mean of all the counts was recorded. Randomly 10 plants from each experimental treatment unit were selected. All the mature panicles were removed from the plants and counted. The average of these counts gave the number of panicles per plant.

Randomly selected 10 plants and using a high precision ruler length of the fully emerged panicle without stalk was measured. Grains per panicle (GPP) were counted by randomly selecting 10 plants, manually threshed, and counted. All counts were averaged. Thousand-grain weight (TGW) [g] was calculated at maturity, the whole experimental plot was cut down, and piles were made. Before harvesting, all the matter was weighed on high precision weighing balance.

After harvesting, all the panicles from the piles were removed and kept air-dried and then threshed for grains. Grain yield was calculated and expressed in kg ha^{-1}. Biomass accumulation (t ha^{-1}) was calculated by adding whole economic yield and vegetative yield. All the above-ground matter of the plants including vegetative and reproductive parts piled up and weighed for biomass accumulation analysis.

Statistical analysis: The laboratory experiment was performed in a completely randomized design with three repeats, while the field experiment was performed in a completely randomized block design with three repeats. For principal component analysis (PCA) and hierarchical clustering, ultimate software for graphing and analyzing the Origin pro-2022 and statistical package R v4.1.2 was used. For lateral statistical analysis and graphs, a graphing and analyzing tool Graph Pad Prism v 9.3.1(471) was used.

RESULTS

Two hundred accessions (Table 1) were tested for germination behavior at optimal controlled temperatures ranging from 18 °C, 25°C, 32°C, and 39 °C. Accessions were grown best at 32 °C and 135 out 200 were germinated 80%. Therefore, 135 accessions were sustained for further study. Means±SE of different germination strength parameters (G%, G_{50}, MGT, MGR, GI, U, Z, CVG) and seedling vigor parameters (RDW, SFW, SDW, RL, and SL) from greenhouse experiment are delineated in (Table 2). The germination percentage of some accessions were found 100% (A12-IN, A40-AnIN, A73-KrIN, A94-RaIN, A95-RaIN, A101-UtIN, A106-UtIN, and A111-SA), while others 80 to 90%. However, most of the accessions which were having 60 or less than 60% germination were not selected for further field study at this time. Detailed descriptive statistics of the germination and seedling vigor parameters are given in (Table 3). Interactively germination percentage (G%) of all the accessions were 83.77, and GI=3.33, MGT=3.26, MGR=0.30, G50=2.62, Un=1.74, Z=0.25, CVG=31.84. Low and up confidence interval, Standard deviation (SD), variance, Coefficient of variation (C.V.), minimum, maximum, median, 1st and 3rd quartile is also given in Table 3 for a complete elaboration of the results.

Seeds of foxtail millet normally germinated in 4 to 5 days to completely protrude out under controlled conditions, while emergence in the field conditions from 6 to 7 days as observed in the field experiment later. For seedling vigor analysis, RL= Root Length (cm), SL= Shoot Length (cm), RDW= Root Dry Weight (g), SFW= Shoot Fresh Weight (g), SDW=Shoot Dry

Table 1. List of successive accessions after germination & seedling vigor test.

Sort#	Accession No.	Lot No.	Year	Country of origin	Coordinates	Plant name
A12	Pl 180483	88ncab01 SD	2004	India	20.5° N, 78.9° E	BHATTI
A13	Pl 180849	9ncai01 SD	2005	Turkey	38.9° N, 35.2° E	KUMDARI
A15	Pl 195753	88ncai02 SD	2004	China	35.8° N, 104.1°E	Y.S. 811
A19	Pl 219619	88ncai01 SD	2004	Pakistan	30.3° N, 69.3° E	---
A21	Pl 220545	88ncab01 SD	2004	Afghanistan	33.9° N, 67.7° E	GAL
A44	Pl 464163	82ncai01 SD	2004	Andhra Pradesh, India	15.9° N, 79.7° E	I.Se 7
A71	Pl 464286	83ncai01 SD	2005	Kerala, India	10.8°N,76.2°E	I.Se 140
A73	Pl 464289	83ncai01 SD	2005	India	20.5° N, 78.9° E	I.Se 42-B
A93	Pl 464459	86ncab01 SD	1998	Punjab, India	31.1°N,75.3°E	I.Se 306-A
A101	Pl 464482	84ncai01 SD	2003	Uttar Pradesh, India	26.8°N,80.9°E	I.Se 329
A106	Pl 464489	87ncai01 SD	1998	Uttar Pradesh, India	26.8°N,80.9°E	I.Se 339
A107	Pl 464514	86ncai01 SD	1998	Assam, India	26.2°N,92.9°E	I.Se 374
A108	Pl 464516	84ncai01 SD	1998	Assam, India	26.2°N,92.9°E	I.Se 376
A111	Pl 464546	84ncai01 SD	1999	South Africa	30.5°N,22.9°E	I, Se 415
A115	Pl 464553	84ncai01 SD	1998	Kenya	0.02° N, 37.9° E	I.Se.505
A118	Pl 464556	84ncai01 SD	1998	Kenya	0.02° N, 37.9° E	I.Se.509

Table 2. Germination and seedling vigor parameters (Means±SE) for selected accessions out of (A1---A135)

ACC.	SEEDLING VIGOR					SEEDLING STRENGTH							
	SL (cm)	*RL*	*SFW*	*SDW*	*RDW*	G%	MGT	MGR(d^{-1})	G50 (d)	GI (d)	CVG%	U (bit)	Z
A4-TR	2.5±0.03	4.4±0.21	4.0±0.01	2.7±0.11	1.15 ±0.01	90±6	3.22±0.12	0.31±0.01	2.5±0.03	4.09±0.03	59.6±7	2.42±0.02	0.11±0.01
A12-IN	3.6±0.14	6.2±0.23	5.2±0.11	3.0±0.02	1.49 ±0.02	100±7	2.6±0.15	0.38±0.02	2.2±0.03	4.92±0.23	45.15±6	1.97±0.01	0.18±0.02
A15-CN	3.6±0.32	6.3±0.09	6.7±0.04	3.9±0.01	1.93 ±0.02	90±9	3.33±0.05	0.3±0.05	2.64±0.03	2.78±0.11	21.21±4	0.99±0.03	0.58±0.01
A19-PK	3.6±0.04	5.9±0.23	2.2±0.01	1.7±0.01	0.63±0.01	90±12	3.22±0.03	0.31±0.06	2.5±0.03	4.09±0.22	59.65±9	2.42±0.05	0.11±0.01
A21-AF	3.7±0.12	5.6±0.11	2.5±0.05	1.5±0.01	0.73±0.01	90±8	4.44±0.11	0.22±0.03	3.75±0.23	2.07±0.22	16.35±4	1.22±0.06	0.44±0.02
A30-PK	3.6±0.05	5.1±0.15	0.4±0.03	0.7±0.01	0.02±0.01	90±6	3.89±0.11	0.26±0.03	3.42±0.23	2.37±0.21	15.45±3	1.22±0.03	0.44±0.03
A40-AnIN	2.9±0.11	4.7±0.18	0.7±0.02	0.5±0.01	0.03±0.01	100±12	4±0.15	0.25±0.02	3.33±0.23	2.84±0.03	39.09±3	2.32±0.02	0.16±0.04
A44-AnIN	3.72±0.32	6.4±0.12	0.8±0.02	0.7±0.02	0.24±0.02	90±18	2.67±0.13	0.38±0.02	2.25±0.33	4.42±0.23	45.93±4	1.97±0.02	0.17±0.02
A71-KrIN	3.7±0.12	6.2±0.24	6.3±0.01	3.8±0.11	1.80±0.05	80±11	2.57±0.12	0.39±0.05	2.17±0.23	3.25±0.26	37.95±10	1.84±0.01	0.19±0.02
A73-KrIN	3.7±0.01	5.6±0.21	6.2±0.22	4.1±0.12	1.79±0.06	100±19	4±0.12	0.25±0.07	3.33±0.33	2.84±0.33	39.09±13	2.32±0.11	0.16±0.05
A80-MpIN	3.6±0.13	5.5±0.21	3.9±0.14	2.6±0.03	1.12±0.07	90±16	3.33±0.12	0.3±0.03	2.25±0.23	3.25±0.36	49.75±12	1.84±0.13	0.22±0.02
A83-TnIN	3.8±0.02	5.8±0.22	3.4±0.11	2.5±0.11	0.99±0.04	80±21	2.62±0.07	0.38±0.03	2±0.02	4.12±0.26	53.63±13	2.25±0.1	0.11±0.01
A84-TnIN	3.5±0.01	5.5±0.05	1.9±0.12	1.6±0.21	0.54±0.07	80±22	2.62±0.07	0.38±0.07	1.67±0.23	4.57±0.26	64.19±5	1.91±0.12	0.18±0.05
A86-TnIN	3.8±0.11	5.8±0.04	3.7±0.14	2.1±0.05	1.07±0.04	80±12	2.57±0.06	0.39±0.02	2.17±0.3	3.25±0.22	37.95±6	1.84±0.02	0.19±0.01
A93-PuIN	3.7±0.21	5.6±0.05	6.8±0.15	3.8±0.03	1.95±0.03	90±8	2.89±0.05	0.35±0.07	2.62±0.123	4.08±0.23	40.38±5	1.53±0.01	0.28±0.03
A94-RaIN	3.8±0.13	5.9±0.09	3.8±0.11	2.5±0.02	1.11±0.07	100+12	2.5±0.11	0.4±0.02	2±0.11	5.42±0.22	50.77±16	1.97±0.02	0.18±0.05
A95-RaIN	3.5±0.09	5.5±0.15	3.7±0.13	2.1±0.07	1.07±0.08	100±15	3.9±0.14	0.26±0.01	3.5±0.23	2.8±0.23	28.22±2	1.85±0.02	0.22±0.05
A96-RaIN	3.5±0.22	5.5±0.19	3.7±0.02	2.4±0.01	1.07±0.03	90±17	3.89±0.16	0.26±0.07	3.42±0.22	2.37±0.25	15.45±6	1.22±0.11	0.44±0.02
A101-UtIN	3.7±0.01	5.7±0.12	6.4±0.01	4.0±0.02	1.83±0.02	100±9	3.4±0.15	0.29±0.02	2.5±0.12	4.43±0.24	60.75±7	2.72±0.23	0.07±0.02
A106-UtIN	3.6±0.51	5.6±0.16	4.2±0.04	2.2±0.03	1.20±0.07	100±10	4±0.12	0.25±0.08	3.33±0.22	2.84±0.25	39.09±8	2.32±0.12	0.16±0.03
A107-AsIN	3.8±0.15	5.9±0.34	2.5±0.11	2.1±0.21	0.72±0.02	90±18	4±0.15	0.25±0.02	3.25±0.21	2.4±0.04	27.95±5	1.84±0.11	0.22±0.022
A111-SA	3.7±0.12	5.8±0.05	7.5±0.13	4.6±0.14	2.16±0.01	100±12	2.6±0.12	0.38±0.02	2.2±0.11	4.92±0.22	45.15±6	1.97±0.11	0.18±0.032
A118-KE	3.5±0.34	5.9±0.04	4.3±0.02	2.7±0.23	1.24±0.08	90±10	3.22±0.12	0.31±0.01	2.5±0.21	4.09±0.21	59.65±7	2.42±0.11	0.11±0.032

Seedling vigor: RL= Root length (cm), SL= Shoot length (cm), RDW= Root dry weight (g), SFW= Shoot fresh weight (g), SDW= Shoot dry weight (g)
Seedling strength: G%= germination (%), MGT (Mean germination time) [Days], MGR= Mean germination rate (MGR) per Day, Un = Uncertainty of germination, Z = Synchrony of Germination Process, CVG=Coefficient of the velocity of germination, GI= Germination index, G50= Time taken to 50% germination (Days)

Table 3. Summary/statistical description of germination strength & seedling vigor of germinable 135 accessions

Parameter	SEEDLING STRENGTH								SEEDLING VIGOR & YIELD					
	G%	MGT (d)	MGR (d^{-1})	G_{50} (d)	GI (d)	CVG%	U (bit)	Z	*SL (cm)*	*RL (cm)*	*SFW (g)*	*SDW (g)*	*RDW(g)*	Yield
Low 95% CI	81.58	3.15	0.300	2.51	3.14	30.78	1.66	0.23	3.64	5.24	2.36	1.70	0.67	**88**
Mean	83.77	3.26	0.310	2.62	3.33	31.84	1.74	0.25	3.67	5.33	2.63	1.85	0.75	**90**
Up 95% CI	85.96	3.37	0.320	2.72	3.52	32.90	1.81	0.27	3.69	5.42	2.90	1.99	0.82	94
SD	12.86	0.64	0.060	0.60	1.11	6.247	0.44	0.12	0.14	0.52	1.59	0.84	0.45	6
Variance	165.4	0.41	0.004	0.36	1.24	39.03	0.19	0.01	0.02	0.27	2.53	0.71	0.20	97
SE Mean	1.10	0.05	0.005	0.05	0.09	0.537	0.03	0.01	0.01	0.04	0.13	0.07	0.03	0.58
C.V.	15.35	19.6	19.54	23.0	33.5	19.61	25.5	48.5	2.06	9.86	60.5	45.6	60.5	26
Minimum	60.0	2.57	0.220	1.67	1.75	22.50	0.99	0.07	3.33	4.10	0.86	0.07	0.80	45
1st Quartile	80.0	2.62	0.260	2.17	2.23	25.64	1.30	0.17	3.56	4.80	1.76	1.16	0.50	94
Median	90.0	3.33	0.300	2.50	3.25	30.00	1.84	0.19	3.68	5.53	2.85	1.91	0.81	96
3rd Quartile	90.0	3.90	0.380	3.20	4.37	38.10	1.97	0.39	3.78	5.69	3.54	2.36	1.01	98
Maximum	100	4.44	0.430	3.75	5.42	42.86	2.72	0.58	3.98	6.39	7.58	4.66	2.16	122

Seedling vigor: RL= Root length (cm), SL= Shoot length (cm), RDW= Root dry weight (g), SFW= Shoot fresh weight (g), SDW= Shoot dry weight (g)
Seedling strength: G%= germination (%), MGT (Mean germination time) [Days], MGR= Mean germination rate (MGR) per Day, Un = Uncertainty of germination, Z = Synchrony of Germination Process, CVG=Coefficient of the velocity of germination, GI= Germination index, G50= Time taken to 50% germination (Days)

Weight (g) are given in Table 3. While shoot length (SL)=4.67±0.01 and RL=5.33±0.04, SFW=2.63±0.13, SDW=1.85±0.07, RDW=0.75±0.03. Low and up confidence intervals, Standard deviation (SD), variance, Coefficient of variation (C.V.), minimum, maximum, median, 1st and 3rd quartile are also given in Table 3 for the complete elaboration of the results.

Principle component analysis (PCA) was performed to find out the suitable clans of accessions and choose a representative accession for further field experimentation through feature reduction. PCA presented in (Fig. 2) sorted out the two principal components PC1 & PC2. Only two eigenvalues were > 1 and explained a variation of 95%, which showed a good relation between some candidates that can be considered in PC1 (74%) and PC2(24%).

The hierarchical cluster analysis shown in (Fig. 3), separated the similar groups in a circular arrangement. One thirty-five accessions could be seen on the disc which were separated into 14 similar groups. Out of each 14 groups, one representative accession with good germination strength and seedling vigor was selected. These fourteen accessions sorted out one from each fourteen clusters were accession A12-IN (origin India), A15-Ch (origin China), A19-PK (origin Pakistan), A21-AF (origin Afghanistan), A44-ApInd (origin India), A71-KrInd (origin India), A73-IN (origin India), A93-IN (origin India), A101-IN (origin India), A106-UpIN (origin India), A107-AsIN (origin India), A111-SA (origin South Africa), A115-KE (origin Kenya), A118-KE (origin Kenya). Panicles of these fourteen accessions are graphically represented in Fig. 4 (pictures taken from a field experiment). The selected fourteen accessions were grown in three different locations.

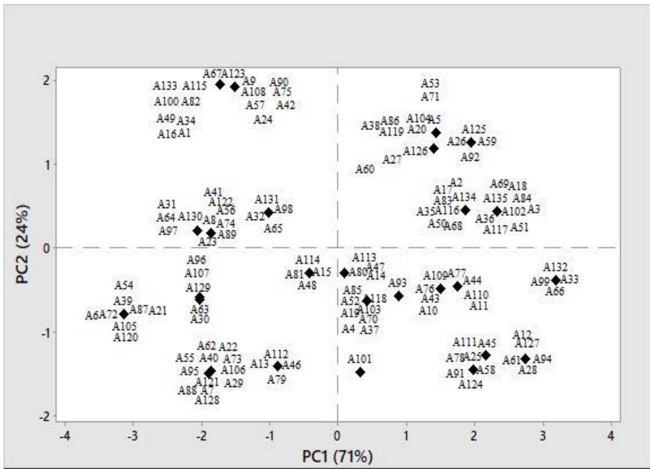

Figure 2. Principal component analysis of 135 successive accessions

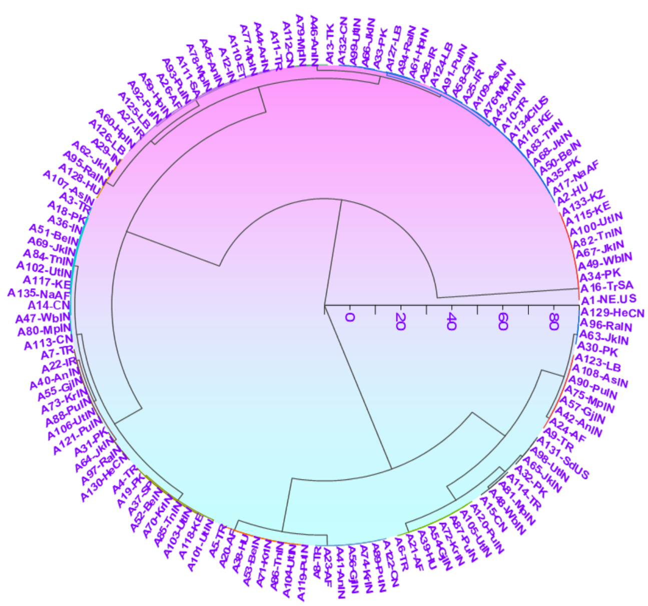

Figure 3. Hierarchial cluster analysis of 135 accessions based on germination strength, seedling vigor and preliminary yield, devising 14 cluster of similar behavior.

Tillers per plant [TPP] were calculated collectively highest in all accessions at the Loc-3 JKA, CJF (well-irrigated 4 to 5 & loamy fertile soil) and it was found at par with location ARA, UAF, while there is a decline in the TPP values for the location DFP, UAF [Figure 4 (a)]. Further, the highest value of TPP=12.3 was recorded for accession A111-SA, followed by accessions A101-IN and A73-IN with the TPP value of 11.3 for both. The same accessions A111-SA, A73-IN, and A101-IN produced 11, 5.7, and 6.7 TPP respectively. Panicles per plant [PPP] [Figure 4 (b)]. The maximum PPP was counted for the accessions at JKA, CJF with the highest value of PPP=12.3 for the accessions A73-IN followed by accessions A101-IN and A107-AsIN with the PPP value of 11.7 for both. PPP values were significantly deficient at the location DFP, UAF, however, values are comparable at the location ARA, UAF.

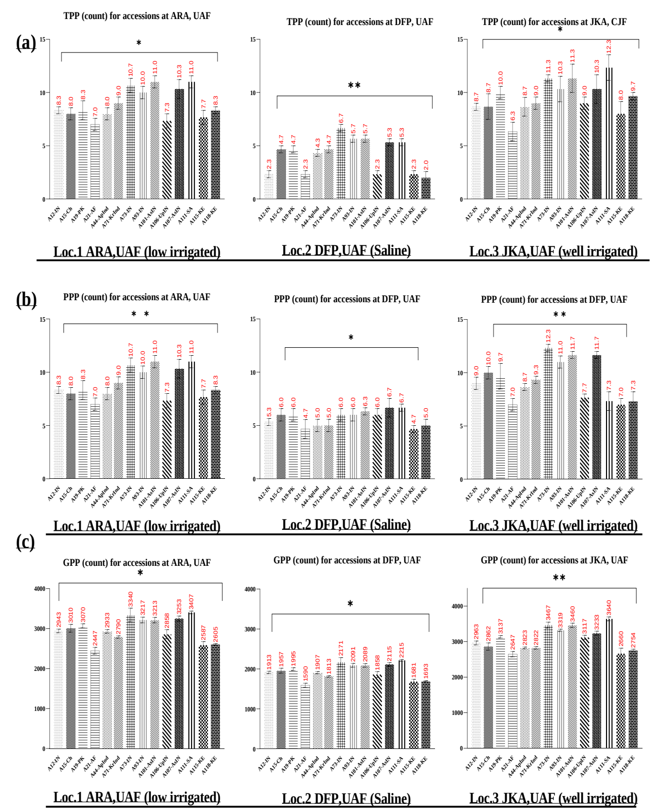

Figure 4. (a,b & c) Performance of selected accessions to produce tillers per plant [TPP], panicles per plant [PPP] and grains per panicle [GPP] at three different locations 1-ARA, UAF; 2-DFP, UAF; 3- JKA, CJF

Grains per panicle [GPP] are given in [Figure 5 (c)]. Maximum GPP were recorded at the location JKA, CJF i.e., well irrigated, and fertile loamy soil condition. Maximum GPP were counted in the accessions A111-SA, A101-IN, and A73-IN with the GPP count of 3640, 3467, and 3460 respectively. GPS at locations DFP, and UAF were significantly lower. GPP for accessions A111-SA, A101-IN, and A73-IN were 2215, 2089, and 2171. However, GPP values at locations ARA, and UAF were non-significantly lower than GPP at JKA, and CJF. GPP for accessions A111-SA, A101-IN, and A73-IN were 3407, 3213, and 3340 respectively.

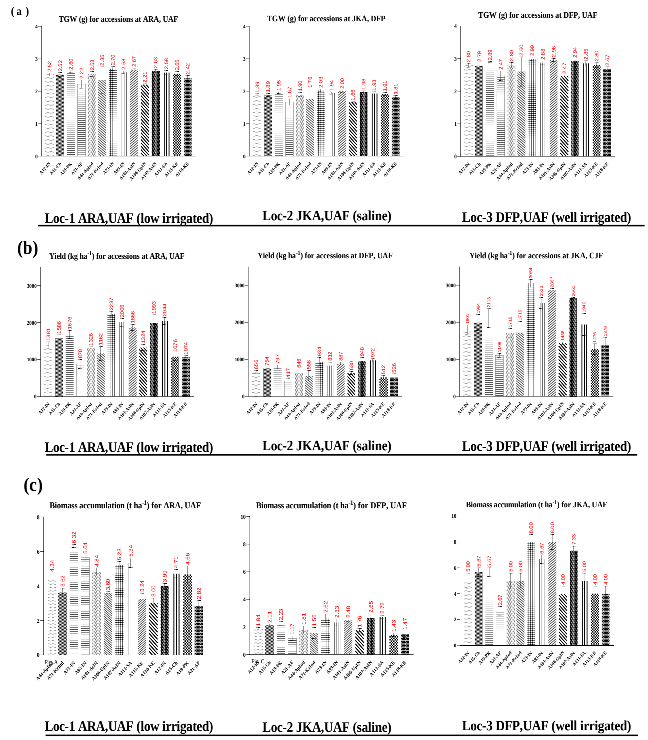

Figure 5. (a, b & c) Performance of selected accessions for Thousand grain weight (TGW) [g], Yield [kg ha^{-1}] and biomass accumulation (t ha^{-1}) in three different locations 1-ARA, UAF, 2-DFP, UAF, 3-JKA, CJF

Maximum, thousand-grain weight (TGW) [g] was recorded for accessions at JKA, CJF followed by ARA, UAF i.e., the statistically non-significant difference [Figure 5 (a)]. TGW was significantly lowered for accessions at DFP, UAF. Maximum TGW was weighed for accessions A73-IN, A93-IN, A101-IN, and A111-SA with the value of 3.0, 2.9, 3.0, and 2.9 at JKA, CJF, and with values of 2.7, 2.6, 2.7 and 2.6 at ARA, UAF; while it was significantly lowered at DFP, UAF with the value of 2.03, 1.94, 2.0 and 1.93 respectively.

Maximum, yield [g/pot] was recorded for accessions at JKA, CJF followed by ARA, UAF i.e., statistically non-significant [Figure 5 (b)]. Yield was significantly lowered for accessions at DFP, UAF. Maximum yield was recorded for the accessions A73-IN, A101-IN and A107-AsIN with the value of 3054, 2867and 2661 kg ha^{-1} at JKA, CJF, and with the value of 2237, 1866, and 2044 kg ha^{-1} at ARA, UAF; while it was significantly lowered at DFP, UAF with the values of 934, 887, and 948 kg ha^{-1} respectively. Accessions A107-AsIN and A111-SA performed comparatively better at DFP, UAF with the yield value of 948 and 972 kg ha^{-1}.

Biomass accumulation [g/pot] is given in [Figure 5 (c)]. It can be observed from the figure that maximum biomass accumulation was recorded for accessions at JKA, CJF followed by ARA, UAF i.e., statistically non-significant. Biomass accumulation was significantly lowered for accessions at DFP and UAF. Maximum biomass accumulation was recorded for the accessions A73-IN, A101-IN, and A107-AsIN with the value of 8.0, 8.0, and 7.33 t ha^{-1} at JKA, CJF and with the value of 5.23, 3.24 and 3.99 t ha^{-1} at ARA, UAF; while it was significantly lowered at DFP, UAF with the value of 2.62, 2.48, and 2.65 t ha^{-1} respectively. The highest biomass accumulation at ARA, UAF was recorded in the accessions A19-PK. Accessions A93-IN, A111-SA, and A115-KE produce comparatively more biomass accumulation at ARA, UAF with the value of 5.34, 4.71, and 4.66 t ha^{-1}. Similarly, A115-KE also produced better biomass accumulation at location DFP, UAF with biomass accumulation of 2.72 t ha^{-1}.

If we compare location JKA, CJF (fertile, well-irrigated) with JKA, CJF (Saline, well-irrigated), and all the accessions reduced yield and biomass, however the somewhat competitive yield is still standing there. Comparing the top yield producing accession A73-IN which produced 3054 kg ha^{-1} in the JKA. CJF under fertile and well-irrigated areas, while the same accession produced 69% less yield of 934 kg ha^{-1} in DFP, UAF under the saline area. Similarly, A73-IN produced biomass accumulation of 8 t ha^{-1} in the JKA, CJF, and only 2.62 t ha^{-1} in DFP, CJF with a 67% decline in the yield. Similarly, other parameters TPP, PPP, GPP, and TGW are also affected by the salinity area. Comparing the area JKA, CJF and ARA, UAF (fertile, low irrigated) yield differences are there but much less than salinity area. High-yielding accession A73-IN produced 2237 kg ha^{-1} which is 26% less than the JKA, CJF. Similarly, biomass accumulation is 34% declined with a value of 5.2 t ha^{-1}. Drought and salinity both reduced the yield and yield contributing factors, but salinity affected more yield.

DISCUSSIONS

Adaptation of plants in the natural environments is a robust mechanism through which it can manage itself in a several type of environments (Li *et al.*, 2021). In a study by Li *et al.*, 2021, high depth resequencing of 312 accessions reveals adaptation of foxtail millet for geographical divergence. In another study, spatial distribution of genetic diversity of 223 core collection accessions was assessed, and they told that foxtail millet has great potential for adaptation in a variety of environments. To utilize the wide collection of germplasm, a core collection (10% of the entire collection) was utilized (Upadhyaya *et al.,* 2009).

Seeds of Setaria (Foxtail) possess various challenges for germination. The germination percentage of 135 accessions was more than 80%. Other seeds may be dormant and have delayed germination due to various factors (Borza *et al*., 2007). In a similar study base, optimum and ceiling temperature for foxtail millet was calculated at 9.3, 37, and 46 respectively (Koocheki *et al.,* 2006; Nejad *et al.,* 2009). So, the Germination test was performed at 18 °C, 25°C, 32°C, and 39 °C for 200 accessions. The optimum temperature for sowing was 32°C and the study results were not much different from Koocheki *et al.,* 2006; Nejad *et al.,* 2009. These accessions were then tested for germination strength, seedling vigor, and preliminary yield evaluation. Germination strength and seedling vigor parameters showed great diversity in the results. The diversity of results confirms the diversity in germplasm collected. This is also due to the different origins of the germplasm from Asia, Southeast Asia, America, and Europe. Genetic diversity is critical for the adaptation of the specie and better breeding program ((Booy *et al*., 2000)

Root length (RL) and shoot length (SL) of the accessions showed variation among foxtail millet germplasm. The importance of root length is well known because they supply water, minerals, and organic compounds to the plant (Sarker *et al*., 2005). The variation in RL and SL was studied by Ceasar *et al.,* 2020 in five low and high-phosphorus-responding foxtail millet genotypes. Shoot fresh weight (SFW) is one of the traits to know the vegetative growth capacity and biomass of the plants (Asekova *et al*., 2016). The minimum shoot fresh weight (SFW) weighed 0.86±0.13 g and maximum 7.58±0.13 g with variance of 2.53. There was a huge variation among shoot fresh weights which confirms the different growth capacities & biomass production of different accessions. In a research project, similar root length and shoot length differences were found among foxtail millet accessions (Ardie *et al.,* 2015). Root dry weight (RDW) is the most sensitive parameter to evaluate various genotypes because it determines the capacity of plants to extract nutrients from the soil for plant growth and development (Sudhalakshmi *et al*., 2007). There was found great range and variability in the RDW depending upon germination and growth strength i.e., minimum RDW weighed was 0.86±0.03 g and maximum was 2.16±0.03 g with the variance of 0.20.

Plant population depends upon the germinability of the seeds and differs among population depending upon heredity and dormancy characters (Baskin and Baskin, 1973). Mean of germination percentage (G%) was calculated 83.77±1.10%, while the minimum and maximum were 60±1.10, and 100% with a variance of 165.4. Twenty-three accessions were found which had 80 or more than 80% G%.

How to evaluate an emergence vigor of a seed lot? Answer to this question is if we take laboratory tests and calculate mean germination time (MGT) and mean germination rate (MGR), these are indirect measurement of emergence vigor in soil. MGT is an indicator of the emergence performance of the seed in the soil and so is the MGR (Matthews and Khajeh Hosseini, 2006). Maximum MGT was calculated at 4.44±0.11 days for accession A21-AF and minimum 2.57±0.12 for the accessionA71-KrIN days were counted. As lesser time positive indication is considered. Similarly, MGR varied from 0.22±0.005 to 0.43±0.005 per day which showed variation in the germination rate of accessions. Similarly, the time taken to 50% germination G50 (days) varied from 1.67±0.05 to 3.75±0.05 days. The germination index (GI) is the degree of speed and percentage of germination (Javaid *et al*., 2018) showed great variability. GI calculation ranged from 1.75±0.09 to 5.42±0.09 days for the 135 accessions from different origin. Higher rate of GI ensures the higher rate of germination (Chachalis and Reddy, 2000). The coefficient of the velocity of germination (CVG) is the index for the speed of germination that determines how speedily a seed will germinate (Rastegar *et al*., 2011). CVG varies from 22.50±0.039 to 42.86±0.039% with a variance of 39.03. Synchrony and Uncertainty (U) of germination estimates how much your germination is scattered red through time and how much is synchronous (Fernández-Pascual *et al*., 2021). Uncertainty of germination varies from 0.99 to 2.72 bit. While synchronization of germination (Z) ranged from 0.07±0.03 to 0.58±0.03. All these seedling strength parameters reveal various aspects of the strength and weaknesses of germination.

It was not possible to compare all the data of 135 accessions which are more or less overlapping each other. So to solve the issue feature reduction technique PCA was used. To reduce variable dimensionality PCA is widely used by researchers (Yang *et al.,* 2021). In the PCA analysis, the first principle component gave a 71% variation, which indicates the similarity in the germination response of various accessions. Further hierarchical clustering was performed, and fourteen different similar clans of accessions were sorted out. Now one accession from each clan with superior germination strength, seedling vigor characteristics and yield were selected for the field study. In a similar study by Upadhyaya *et al.,* 2009, Twenty-nine clusters were formed in a study on 12 quality traits of foxtail millet after PCA to utilize diversity of core collection.

Accessions A12-IN, A15-Ch, A19-PK, A21-AF, A44-ApInd, A71-KrInd, A73-IN, A93-IN, A101-IN, A106-UpIN, A107-AsIN, A111-SA, A115-KE, and A118-KE of different origin were selected for field study showed variance and significance in yield parameters. Most of the accessions e.g. A44-ApInd, A71-KrInd, A73-IN, A93-IN, A101-IN, A106-UpIN, and A107-AsIN were of Indian origin that was found with superior yield traits. There is not much difference between the climates of India and Pakistan and foxtail millet is a genetically diverse specie with a wide range of environments adopted (Seghatoleslami *et al.,* 2007, 2008).

Origin of the accession might play a role viz. eight accessions of Indian origin, one Pakistan, one China, one Afghanistan, one South Africa, and two Kenya.

The yield and yield contributing traits like straw yield, thousand-grain weight, panicle length and breadth, and panicle weight are important traits to be investigated (Yang *et al*., 2020). Yield contributing traits e.g. tillers per plant [TPP], panicles per plant [PPP] and grains per panicle [GPP] at three different locations 1-ARA, UAF; 2-DFP, UAF; 3- JKA, CJF were calculated in this research project with good range in all three locations which confirm wide adaptability of foxtail millet in fertile well-irrigated soil (JKA, CJF) with 3054 kg ha^{-1} yield and in low irrigated soil (ARA, UAF) with 2237 kg ha^{-1} yield as well as in the saline conditions (DFP, UAF) with 934 kg ha^{-1} yield. However, DFP, UAF (Saline area) reduced yield (69%) and biomass (67%) in this study, while in another study by Krishnamurthy *et al*., 2014 core collection of foxtail (n=155) was evaluated against saline and non-saline conditions and found much variability in the genotypes with 30% overall reduction in yield and biomass due to salinity. The yield & yield components (TPP, PPP, GPP & TGW) showed great potential of the foxtail millet under Faisalabad climatic conditions. Higher yields of the accessions may be attributed to the C4 cycle of this crop which is an efficient fixer of carbon dioxide and evolutionary close to major crops like maize, sorghum, and rice (Yang *et al*., 2020). According to Liu *et al.,* 2020, the foxtail millet as a food and forage crop has good productivity. However, very little work on these traits for the crop foxtail millet has been carried out until now, especially almost no work in Faisalabad, Pakistan. Comparing three millet species; pearl millet, proso millet, and foxtail millet. Seghatoleslami *et al.,* 2007 calculated 1.8 tillers per plant only, however, in this study minimum of 6 tillers/plant were observed which showed the greater potential of the crop in the subject area. In an experiment of the genetic diversity of foxtail millet in rainy and post-rainy seasons it was found 10.87 TPP in rainy season and 25.5 TPP in post rainy season (Brunda *et al.,* 2015). Panicle development is prime contributor of yield and crop productivity (Xiang *et al.,* 2017). A study by (Sapkota *et al.,* 2016) showed a great variance among different accessions, however they concluded that high yielding foxtail millet accessions may be selected by indirect selection for their high-yielding parameters (flag leaf length, flag leaf breadth, peduncle length, number of panicles per square meter). In this way, they found that accessions Hamla-149 showed high performance for yield and yield-relating components, and resultantly, this accession may be selected for further evaluation. In a nutrient management study on foxtail millet (Ojha *et al.,* 2018), at 60:30:20 NPK / ha, the highest straw yield (12.6 t ha^{-1}), biomass accumulation (15.02 t ha^{-1}), 88.6 effective panicles, 91 days to maturity, 157.9 cm height, thousand-grain weight (2.44 g), yield (2.46 t ha^{-1}) were estimated. The foxtail millet adaptation study is the revival of the orphan millet crop which is a highly nutritious and biotic and abiotic stress-resistant crop. Out of 200 hundred accessions initially, 14 more robust accessions were selected for the field study in three different environments. Foxtail millet provided comparable results with the world in the local environment of Faisalabad.

***Conclusions*:** The study concluded that foxtail millet might be successfully adapted in the climate of Faisalabad, Pakistan based on shorter duration and higher yield & biomass results found. However, further genome assisted study is needed to find out suitable varieties to cultivate. Additional accessions/genotypes selection is also required against the abiotic and biotic stresses and plant protection studies if any pest or disease is found on the crop, however during the study no disease or pest is observed on the crop. An inquiry in depth is required for the suitability of foxtail millet in the saline areas of Punjab because, in this research project, the yield has declined up to 69%, although there was found variation among the genotypes. A suitable saline tolerant/resistant genotype needed to be inquired out of the genetic diversity found in the germplasm collected. In addition, foxtail millet may be evaluated further in other Punjab areas for food security purposes.

Conflict of interest: The authors declare that there is no conflict of interest.

Author's contribution statement: All authors contribute to conceptualization, however, MT conducted complete research design & methodology and AW supervised it.

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