

MULTI-ENVIRONMENT EVALUATION OF FOXTAIL MILLET ADVANCED LINES FOR FORAGE YIELD STABILITY

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Foxtail millet (*Setaria italica* L. P. Beauv.) is a very important crop in the arid and semi-arid areas. In the present study, five advanced lines and one commercial check (Bastan) were tested across 12 (six locations and two years) environments. An attempt was made to identify superior line(s) with emphasis on high forage yield potential and yield stability over multi-environment trials using GGE biplot analysis. The study was conducted using a randomized complete block design with four replications. Combined analysis of variance for forage yield showed that the genotypes, environments, and the interaction effects were highly significant ($P \leq 0.01$). The environment, genotype and interaction effects accounted for 76.38%, 6.97% and 8.92% of the total forage yield variation, respectively. GGE biplot analysis showed that G5 has both high forage yield and stability across the studied environments and E3 and E4 were high-yielding environments in this study. Which-won-where study partitioned the testing locations into two mega-environments with G5 and G3 as winning genotypes in the first and second mega-environment, respectively. According to discriminate ability and representativeness, the E4 and E12 environments were perfect environments. Overall, existence of extensive crossover interaction clearly suggested that efforts are necessary to identify location-specific genotypes over multi-environment trials for release of new varieties.

Keywords: Cross over interaction, Forage yield, Stability analysis, Foxtail millet, GGE biplot

INTRODUCTION

According to a new United Nations report, the world's population is expected to reach 9.8 billion by 2050. It has also predicted that nearly all this population growth will occur in

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developing countries. Beside urbanization, income level will increase, and thus luxurious lifestyles will be accelerated. Accordingly, in order to feed this larger, more urban and richer population, food production must rise by 70%, a challenge that requires enormous changes in agriculture and the economy, especially in rural regions all over the world (WISE, 2013). Furthermore, climate change is another big issue threatening agricultural sustainability and human food security. On the other hand, environmental stresses have become a global concern for agricultural land and food production systems (LIU *et al.*, 2011). Almost all databases and model-simulated data estimate serious drought across the world in the twenty-first century (ZHAO and DAI, 2015). Thus, it's expected that drought and heat tolerant varieties (such as millets, sorghum and etc.) will most likely be commercially used in the future.

Foxtail millet (*Setaria italica* L. P. Beauv.) is one of the oldest cultivated crops beginning around 5000 to 6000 B.C in China. With C₄ photosynthesis, mechanism would have increased photosynthesis efficiency. In addition, due to its resistance to warm weather, drought stress and high tolerance to poor and low input soils, this crop is widely grown in Asia, Europe, America, Australia, and North Africa (SREENIVASULU *et al.*, 2004; AUSTIN, 2006). Furthermore, being a self-pollinated crop with short life cycle (up to 15 weeks from seed to seed), having small genome size (490 Mbp) similar to developed crops such as rice and several bioenergy kinds of grass such as switchgrass (*Panicum virgatum*) and napiergrass (*Pennisetum purpureum*) make it a suitable model crop for different genetics, genomics and physiologic studies (DOUST *et al.*, 2009; LIU *et al.*, 2011). Foxtail millet grains are rich in starch, proteins and lipids as well as vitamins and minerals, which make it a good source of nutrients in the human diet in many parts of the world, especially in Africa, China and India where food security has always been the primary concern (BAI *et al.*, 2008). Foxtail millet grains contain proteins with higher quality compared to other cereal crops such as rice and wheat as it contains seven of the eight essential amino acids (ZHANG and ZNANG, 2007). Also, foxtail millet could be a good choice when high-quality forage (more than 16 MJ kg⁻¹ DM gross energy) and a short- season emergency hay crop is required (PEIRETTI and TASSONE, 2016). Nonetheless, foxtail millet is a minor crop in the world and has a small contribution to agricultural economics. According to FAO statistics, foxtail millet production data are not available, and data are generally grouped with other millet species. It has been reported that millets (taken together) grain production was about 28 million tons in 2016.

The development of high yield varieties has always been the ultimate objective of crop breeders, especially those who focus on orphan crops. Although researches on the development of high yield varieties have led to release of a large number of new varieties in different crops, genotype by environment interaction (G×E) causes genotypes failure to keep high performance in all environments (MOHAMMADI and NADER MAHMOODI, 2008). So, yield stability has been considered as important as the yield potential in plant breeding and will be of particular concern by continuing changes in climatic condition (FASAHAT *et al.*, 2015). The G×E affects all aspects of the decision making process in crop breeding programs including allocation of resources and funding to breeding programs, germplasm selection, evaluation of germplasm and breeding strategy (DE LEON *et al.*, 2016). Several studies have been undertaken to determine the importance of GE. It has been reported that genetic improvement contributes only 50% of total yield increase for major crops during the past 60 years, whereas the improved management and

cultural practices account for the rest of the increase (SILVEY, 1981; SIMMONDS, 1981; DUVICK, 1996). Wheat yield data collected between 1946 and 1977 in England (mean yield for 1946, 2.4 t ha⁻¹ and for 1977, 4.7 t ha⁻¹) showed that increase in yield during this period of time could be attributed to effects of environmental factors (40-60%), genetic improvement (25-40%) and G×E interaction (15-20%) (KANG, 2002). In order to deal with G×E interaction in breeding programs and separating yield potential and yield stability in multi-environment trials, numerous stability analysis methods including variation coefficient, linear regression, Wricks equivalence (W_i), stability variance of Shukla (σ_i^2), additive main effect and multiplicative interaction (AMMI) and genotype and genotype by environment interaction (GGE) biplot have been proposed (GAUCH and ZOBEL, 1988; ZHANG and KONG, 2002). In the recent decade, AMMI and GGE statistical analysis have been widely used in different studies (RAKSHIT *et al.*, 2012; BOSE *et al.*, 2014; HONGYU *et al.*, 2014; TEMESGEN *et al.*, 2015; ZHANG *et al.*, 2016). Although AMMI analysis provides information on main and interaction effects, interaction effects have been only taken in to account in AMMI analysis and genotype effects have been ignored (ZHANG *et al.*, 2016). In addition, AMMI analysis requires a greater number of genotypes and several years for evaluation in comparison to GGE Biplot, moreover, it makes misleading in identifying which-won-where (YAN *et al.*, 2007; FASAHAT *et al.*, 2015). The phenotypic value is a cumulative measure of genotype main effect (G), environment main effect (E) and genotype by environment interaction effect (G×E). Therefore, for stability analysis, both G and G×E must be considered simultaneously (YAN and TINKER, 2006). GGE biplot integrates the G with G×E interaction and effectively detects stable varieties in the multi-environment dataset. Besides, GGE biplot analysis is not sensitive to a number of genotypes so that it produces the most reliable estimates for evaluation of a small number of genotypes (ROSE *et al.*, 2008). Although, GGE biplot has been carried out on wide range of crops including maize (FAN *et al.*, 2007), wheat (ROOZEBOOM *et al.*, 2008), cotton (XU *et al.*, 2014), rice (SAMONTE *et al.*, 2005), soybean (YAN and RAJCAN, 2002), oat (YAN *et al.*, 2010; ZHANG *et al.*, 2010), sorghum (RAO *et al.*, 2011), proso millet (ZHANG *et al.*, 2016) and so on, application of this analysis on foxtail millet has been limited to a few cases. In this study, we have collected forage yield data of many foxtail millet advanced lines from the national regional test in Iran from 2015 to 2016 and aimed to determine forage yield stability and adaptability of foxtail millet advanced lines and identification of superior line(s) with emphasis on high forage yield potential and yield stability over multi-environment trials.

MATERIAL AND METHODS

Plant material

Five advanced lines (Foxtail millet (*Setaria italica* L. P. Beauv.), developed through pure line selection from a diverse population for 7 years based on early maturity (70-80 days to maturity) and forage yield indices in Seed and Plant Improvement Institute (SPII), Karaj, Iran, and one commercial check cultivar (Bastan) were grown for two years (2015-2016) in six different locations that were representative of divergent climatic conditions in Iran. Each year and location were considered as a specific environment and coded to justify 12 test environments (Table 1 and 2).

Table 1. List of genotypes

No.	Lines	Code	Breeding Institute
1	Kfm/93-3	G1	Seed and Plant Improvement Institute (SPII)
	Kfm/ 93-7	G2	Seed and Plant Improvement Institute (SPII)
3	Kfm/ 93-10	G3	Seed and Plant Improvement Institute (SPII)
4	Kfm/93-14	G4	Seed and Plant Improvement Institute (SPII)
5	Kfm/93-17	G5	Seed and Plant Improvement Institute (SPII)
6	Bastan (Control)	G6	Seed and Plant Improvement Institute (SPII)

Table 2- Characteristics of used environments

Locations	Years	Environment Code	Latitude	Longitude	Altitude (m.a.s.l)	Average Temperature (°C)	Average Rainfall (mm)
Gonbad-e-kavous	2015	E1	37° 20' N	55° 25' E	38	17.8	363
	2016	E2					
Karaj	2015	E3	35° 48' N	51° 00' E	1312.5	14.2	256
	2016	E4					
Varamin	2015	E5	35° 32' N	51° 46' E	924	16.9	156
	2016	E6					
Yazd	2015	E7	32° 00' N	55° 00' E	1216	18.9	55
	2016	E8					
Sari	2015	E9	36° 00' N	53° 40' E	43	16.7	690
	2016	E10					
Birjand	2015	E11	32°53' N	59° 13' E	1462	17	129
	2016	E12					

Experimental design

The experimental design was a randomized complete block (RCBD) with four replications. Each plot consisted of six rows 6 m long and 0.6 m row width. Seed sowing was carried out based on millets optimum soil temperature (15-20°C) and climatic conditions of studied environments (HILLS and PENNY, 2005). Fertilizers were applied according to soil nutrient fact sheet in each environment. Agronomic practices were performed according to local developed methods and data were collected from four middle rows for number of tillers per plant, number of leaves per plant, days to 50% flowering, height, fresh forage yield, dry forage yield and seed yield. Height, tillers and leaves number was measured from ten plants in each plot and averaged measurements were analyzed. Days to 50% flowering of lines were recorded as flowering time. Forage and seed yield data were based on plot average.

Statistical analysis

The collected data were subjected to normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests) and subsequent analyses were performed using normal data. Combined analysis of the data and mean comparison using Duncan's method were done by IBM SPSS Statistics v.22. In the analysis, the genotype was entered as a fixed effect and environments were entered as a random effect. The main effect of the environment was tested against the replication within the environment (R/E), the main effect of genotype was also tested against G by E interaction, and G by E interaction was tested against pooled error. Since the lines were targeted for producing more forage yield in the breeding program and considering that almost whole millet plants are used as forage in Iran, therefore, forage yield was conceded as the end product and was subjected to stability analysis.

The base of GGE biplot has been described in detail in the literature (YAN and KANG, 2003) using the following equation:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{1j} + \lambda_2 \xi_{i2} \eta_{2j} + \varepsilon_{ij}$$

Where Y_{ij} is the performance of i th genotype in j th environment, μ is the total mean, β_j is the main effect of j th environment, λ_1 and λ_2 are the singular values of first and second largest principal components respectively, ξ_{i1} and ξ_{i2} are the eigenvectors of genotype i for PC₁ and PC₂ respectively and η_{1j} and η_{2j} are the eigenvectors of environment j for PC₁ and PC₂, respectively.

Partitioning of PC1 and PC2 eigenvectors to genotype and environment eigenvectors was done using the following equations:

$$g_{il} = \lambda_1^{f_l} \xi_{il} \quad \text{and} \quad e_{lj} = \lambda_1^{1-f_l} \eta_{lj}$$

Where l refers to the principal component number and f is the partition factor. 0 and 1 values were used for partition factor (f_l) in the genotype-focused scaling and environment-focused scaling, respectively.

RESULTS

Combined analysis of variance for measured traits (number of tillers, number of leaves, days to flowering, height, fresh forage yield, dry forage yield and seed yield) of the six genotypes grown across 12 different environments showed that environment main effect and genotype by environment interaction effect were highly significant ($P \leq 0.01$) for all studied traits. The main effect of genotype was significant at 1% probability level for all traits except for number of leaves, days to flowering and plant height (Table 3). The coefficient of variation (CV) ranged from 0.05 for days to flowering to 0.14 for seed yield. The relative contribution of environment, genotype, and genotype by environment interaction indicated that environment was the most important source of variation for all studied traits except for number of tillers and days to flowering. Variance component by environment ranged from 29.05% for number of tillers to 80.06% for seed yield. The maximum and minimum contribution of genotype variation source

was 25.56% for number of tillers and 2.22% for seed yield, respectively. In addition, genotype by environment interaction was at the maximum (45.23%) for days to flowering and minimum (6.48%) for seed yield traits (Table 3). In case of fresh forage yield, genotype variance accounted for only 6.97% of total variance, whereas the contribution of environment and genotype by environment interaction were found to be 76.38% and 8.92%, respectively.

Table 3. Combined analysis of variance for studied traits based on six locations and two years

S.O.V	d.f	MS						
		Tiller No.	Leaves No.	Days to flowering	Height (Cm)	Fresh forage yield (t ha ⁻¹)	Dry forage yield (t ha ⁻¹)	Seed yield (t ha ⁻¹)
Environments	11	15.913**	16.95**	537.787**	6450.86**	1532.08**	159.944**	19.951**
Replications within environment	36	0.286	0.632	23.481	118.174	11.711	1.938	0.197
Genotypes	5	30.801**	2.664 ^{ns}	337.205 ^{ns}	1188.494 ^{ns}	307.77**	28.832**	1.221**
Genotypes× Environment	55	4.093**	2.824**	157.183**	527.468**	35.789**	3.160**	0.323**
Error	180	0.206	0.360	11.154	56.621	6.486	0.839	0.126
Variance component by environment (%)		29.05	42.09	30.95	58.86	76.38	76.55	80.06
Variance component by genotypes (%)		25.56	3.00	8.82	4.92	6.97	6.27	2.22
Variance component by interaction (%)		37.36	35.06	45.23	24.06	8.92	7.56	6.48
CV		0.10	0.06	0.05	0.07	0.10	0.13	0.14

ns, * and ** indicate non-significant, significance at 5% and 1% respectively.

The comparison of means and genotypes ranking results are presented in Table 4. Average forage yield of G6 (commercial check variety), across all environments, was 23.26 t ha⁻¹. Forage yield of all studied genotypes, except for G4, was higher than check variety. The overall mean was 24.75 t ha⁻¹ and an average yield of G3 and G5 was over this cutoff, whereas only G5 was significantly different from other genotypes (Table 4).

The average environmental forage yield across genotypes ranged from 8.28 t ha⁻¹ in E11 to 34.68 t ha⁻¹ in E3. Moreover, E3 and E4 were the highest-yielding environments and E11 and E12 were classified as the lowest yielding environments (Table 4). The check variety (Bastan) followed by G5 ranked as the first in one of the 12 environments (E1). Similarly, the best performing genotype (G5) was the first in eight environments (E1, E4, E5, E6, E7, E9, E11, and E12), however, G4 couldn't reach the first rank in any environments (Table 4).

Table 4. Means comparison and ranking of studied genotypes in different environments.

	G1	G2	G3	G4	G5	G6 (check)	Means*	Rank
E1	17.58	27.92	22.83	22.46	<u>28.21</u>	<u>28.21</u>	24.53 ^d	8
E2	22.72	<u>23.65</u>	23.57	13.00	22.17	19.59	20.78 ^e	10
E3	35.85	29.40	<u>42.70</u>	29.23	38.70	32.18	34.68 ^a	1
E4	30.08	29.08	36.40	30.05	<u>41.88</u>	33.10	33.43 ^{ab}	2
E5	32.67	30.77	30.14	30.88	<u>36.78</u>	30.43	31.94 ^b	3
E6	28.67	28.67	27.36	27.60	<u>34.59</u>	31.23	29.69 ^c	4
E7	27.40	26.73	23.22	22.80	<u>33.60</u>	22.65	26.07 ^d	5
E8	<u>27.94</u>	24.54	19.79	21.84	27.72	22.25	24.01 ^d	9
E9	25.55	24.38	26.05	27.75	<u>29.20</u>	22.48	25.90 ^d	6
E10	23.96	20.83	<u>31.36</u>	25.21	31.13	22.61	25.85 ^d	7
E11	8.38	6.75	10.00	7.25	<u>10.45</u>	6.83	8.28 ^e	12
E12	10.30	10.02	14.22	10.63	<u>18.60</u>	7.63	11.90 ^f	11
Means	24.26 ^b	23.56 ^b	25.64 ^b	22.39 ^b	29.42 ^a	23.26 ^b	24.75	
Rank	3	4	2	6	1	5		

* Genotypes with at least one same letter are not significantly different.

The ranking of genotypes and environments based on forage yield and stability indicated the first two PC accounted for 73.31% of total variation (Fig. 1). The genotypes and environments are placed on the right of the AEC on vertical axis indicating higher yield than average yield. Accordingly, the biplot sorted the six foxtail millet genotypes as G5> G3> G1> G2> G6> G4. Moreover, the biplot organized the studied environments based on the forage yield as E4> E3> E12> E7> E10> E5> E6> E2> E9> E8> E11> E1. Genotypes with strong stability have small line length perpendicular to horizontal AEC axis (Fig. 1) (LAKEW *et al.*, 2014). Therefore, G5 and G1 showed the best stability and G3 revealed the maximum fluctuation among the studied genotypes. Considering the yield of all genotypes across all environments, E2 and E9 were found to be relatively stable environments, however not as high-yielding environments (Fig. 1b). To identify the which-won-where pattern and interaction pattern between genotypes and environments, the polygon view of a biplot was fitted to the data and superior genotypes in a specific environment were identified (Fig. 2). Polygon view of foxtail millet genotypes indicated four sections for the studied genotypes and two mega-environments. The first mega-environment consisted of E1, E2, E4, E5, E6, E7, E8, E9, E11, E12 and the second mega-environment was comprised of E3 and E10. The G2, G3, G4, and G5 were the vertex genotypes and were located at the farthest distance from the biplot origin. These terminal genotypes were either the best or the poorest genotypes in some or all environments (TARIKU, 2017).

From plant breeding point of view, an ideal genotype must have high yield potential and yield stability (YAN and TINKER, 2006). In GGE biplot, such an ideal genotype are drawn graphically as the smallest circle in the center and the distance of real genotypes is compared with it and closer genotypes to ideal genotype are preferred. From the results, G5 genotype placed at the nearest distance from ideal genotype and was more favorable. The order of other

studied genotypes according to ideal genotype was G3> G1> G6> G2> G4 (Fig. 3a). In GGE biplot, the angle of any two environments represents the correlation between environments as indicates a positive correlation for acute angle, negative correlation for obtuse angle and no correlation perpendicular angle (YAN and KANG, 2003). Accordingly, there were positive correlations among all environments. There was an intensive correlation among E4, E12, E2, E9, E11 and E5 environments while E1 and E3 were the farthest environments (Fig. 3b).

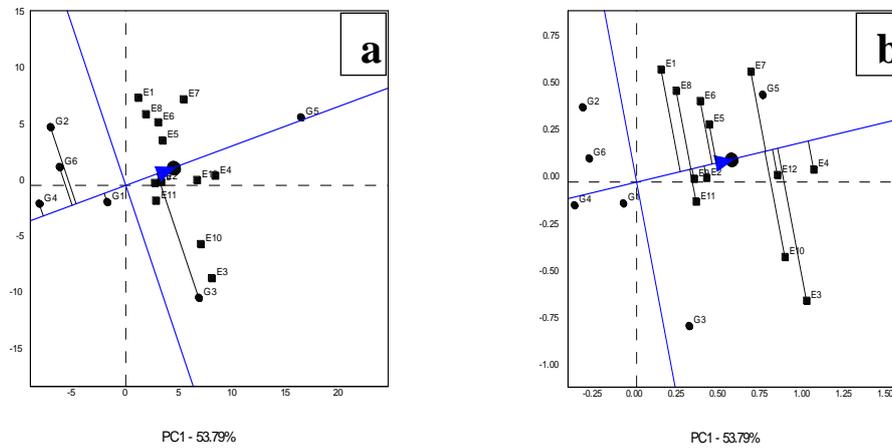


Figure 1. GGE biplot showing the ranking of genotypes (a) and environments (b) for both yield and stability performance

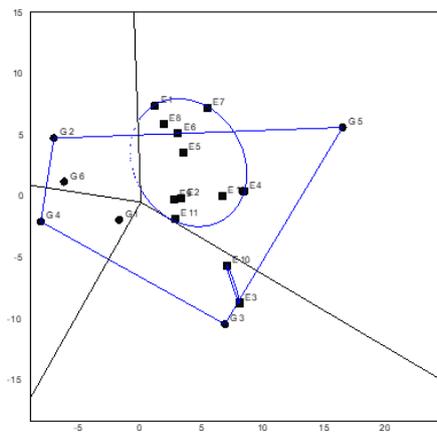


Figure 2. The which-won-where view of the GGE biplot

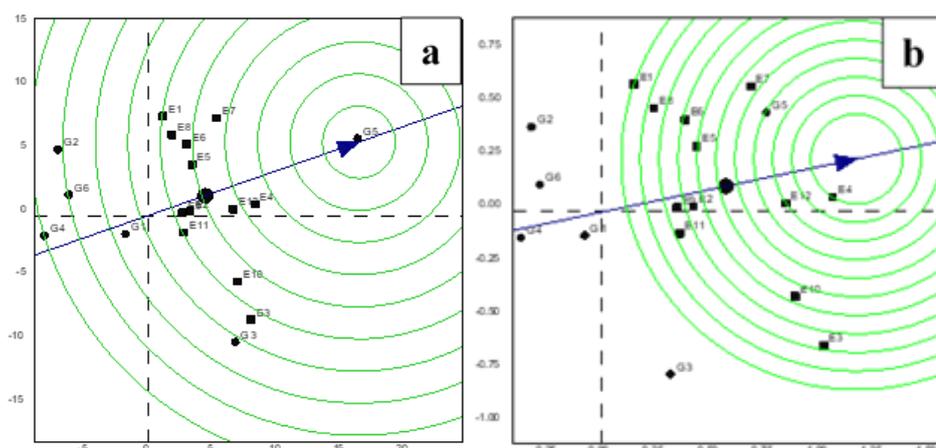


Figure 3. GGE biplot of ideal genotype and comparison of the genotypes with the ideal genotype (a). Discriminating and representativeness environments and correlation between the test environments (b)

In the stability analyses, the value of each environment is determined by discriminating ability and representativeness of the target environment (TARIKU, 2017). Similar to an ideal genotype, an ideal environment is defined as a small circle in biplot which depends on all studied environments performance. In GGE biplot view, an environment discrimination ability and vector length of environment correlate positively whereas the distance of environment vector with ideal environment negatively correlate with the representativeness of the target environment. From fig 3b, the discrimination ability of studied environments was in the order of E4> E12> E7> E10> E5> E2 and E6> E9> E3 and E11> E8> E1. Furthermore, the rank of environments for being representative environment was E9 and E2> E12> E4> E11> E5> E6 > E7> E8> E10 > E1> E3.

DISCUSSION

Plant breeders often perform multi-environment experiments over years to investigate the performance of test entries to provide a strong basis for detecting and developing stable varieties. The total sum of squares for the obtained data from such experiments can be divided into assignable and non-assignable (residual) causes, where assignable causes are from three main sources: the genotype main effect, the environment main effect, and genotype by environment interaction. Genotype by environment interaction (GEI) is the most important challenge for plant breeders, geneticists, and agronomists as well as farmers due to its worldwide presence and consequences. Plant breeders focus on GEI because GEI plays as a confounding factor in cultivar selection plans and affects all aspect of decision making in breeding programs (DAWSON *et al.*, 2011; MIKÓ *et al.*, 2014). Interpretation of GEI and stability detection of cultivars is important in recommending and selecting the cultivar for different ecological regions. GGE biplot is a

powerful methodology to analyze multi-environment trial (MET) and to dissect complex interaction effect (YAN and TINKER, 2006). It can effectively determine interaction pattern graphically, illustrate which environment is preferred by a cultivar and visualize the stability of cultivars in the studied environments besides identifying and drawing the mega-environments among the different locations (YAN *et al.*, 2007). In the current study, we employed this robust method to analyze the GEI in foxtail millet and we showed the importance of genotype, environment, and genotype by environment interaction in foxtail millet MET data. The significance of genotype performance across different environment was related to sufficient genetic diversity among foxtail millet germplasms. The significance of the environment main effect of and the magnitude of variance created by the testing environments indicated the difference between the environments in terms of geographical features (such as altitude) and climatic conditions (such as rainfall, rainfall distribution, temperature and etc.) (KANG, 1997). Based on ANOVA analysis, the effect of GEI on all studied traits was highly significant suggesting that response of cultivars in different environments does not change in the same way and is influenced by the particular conditions of the environment (Table 3). Moreover, environment contributed 29.05-80.06 of the variation in the data and was the most important source of variation for most of the studied traits. Environment effect was more than 10 and 8 times of genotype effect and interaction effect for fresh forage yield, respectively (Table 3). Such a large variance component by the environment is common in MET data and has been reported by previous authors (GAUCH and ZOBEL, 1988; PUTTO *et al.*, 2008; RAKSHIT *et al.*, 2012; LAKEW *et al.*, 2014). In this study, GEI was much higher than genotype effect as shown in table 3. This result suggests that there is a marked GEI in this experiment and selection and recommendation of cultivars for general or specific region needs to study stability and adaptation pattern of genotypes (TEMESGEN *et al.*, 2015). Moreover, the magnitude of GEI variance in compared with genotype variance shows possible existence of different mega-environments in testing locations (YAN and HUNT, 2002).

MET data may include a combination of crossover and non-cross over types of GEI (TARIKU, 2017). Crossover interaction is one of the worst complexity in breeding programs and indicates the change in yield ranking of genotypes across the environment while non-cross over interaction doesn't affect the genotypes ranking in MET data (MATUS-CADIZ *et al.*, 2003). From table 4, the inconsistency of forage yield ranking of genotypes across different environments showed the presence of crossover GEI. However, G5 genotype kept its first ranking under eight environments and indicated that not only cross over GEI presents in these data but also non-cross over GEI plays an active role in GEI nature.

In GGE biplot, the complex GEI are dissected in different PC and are organized graphically against different PC (YAN and TINKER, 2006). The horizontal axis (PC1) showed the main effects of the genotypes and the vertical axis (PC2) expressed the interaction effects as shown in Fig.1 (YAN, 2002). The single arrow line passed from the biplot origin to the average environments, indicated by a black filled circle which is refers to average environment coordinate (AEC) axis (YAN and KANG, 2003). The biplot adequately estimates the GEI if the first two PC justify more than 60% of total variance in data and genotype and interaction effects contributed more than 10% of the total variance (YAN *et al.*, 2010). In this study, the first two PC explained 73.31% of total variation and genotype accompanied with interaction effects

accounted for 15.89% of total variance for fresh forage yield (Table 3) and this method could safely apply to interpret of the variability in these data. The projection of genotypes based on genotype scaling method to PC1 and PC2 in fig. 1 showed more stable genotypes have poor yield while G5 was an exception. G1, G2, G4 and G6 were on the left side of the coordinate axis, it means these genotypes were the poorest yielders with good stability while G3 was the most unstable genotype. GGE biplot analysis showed that G5 has both high forage yield and stability. Performances of G5 was better in most locations (Table 4) suggesting that this genotype suited for popularization. Although integration of stability and high performance in a specific genotype is difficult, some authors have identified stable and high performing genotypes in different crops such as barley (DEGHANI *et al.*, 2006), wheat (KAYA *et al.*, 2006), lentil (SABAGHNIA *et al.*, 2008) and rapeseed (DEGHANI *et al.*, 2008).

A good agreement was observed between the order of genotypes performance from GGE biplot and simple arithmetic mean method as shown in table 4. However, inconsistency between GGE biplot and simple arithmetic mean method for the environment were observed. From GGE biplot (Fig. 1), E12 was found to be a high-yielding environment after E3 and E4 while E12 was found to be a poor environment from the simple arithmetic mean method (Table 4). This inconsistency is due to this fact that arithmetic mean to take into account phenotypic value of genotypes (genotype, environment and interaction effect) while environment effect is not involved in GGE biplot analysis and this method displays only genotype and interaction effects (ZHANG *et al.*, 2010).

Presence of cross over interaction in this study resulted in grouping all the environments into mega-environments (TARIKU, 2017). The G5 and G3 genotype were the best performing genotypes in the first and second mega-environments, respectively. The G1 and G6 genotypes, which located near the origin of biplot, were not sensitive to environment changes (BAXEVANOS *et al.*, 2008) and G2 and G4 vertex genotypes, which didn't fall in any environments, were not the winner in any environments so that they were likely faint genotypes in some or all the environments. Many efforts have been made to identify mega-environments and determine specific adopted genotypes in such mega-environments (BLANCHE and MYERS, 2006; NAVABI *et al.*, 2006; LUO *et al.*, 2015; YAN *et al.*, 2015; KRISHNAMURTHY *et al.*, 2017). This is highly essential to develop narrow adopted genotypes for the management of diverse climate region and reduce the cost of breeding programs. Nevertheless, the mega-environment pattern requires being verified through multi-year and -environment experiments. In addition, repeatable which-won-where pattern over years is an essential and adequate condition for mega-environment delineation (YAN *et al.*, 2007; PUTTO *et al.*, 2008). Although it is likely to achieve repeatable inferences as long as we use the same locations and genotypes for testing across years to the extent possible, that could be through an academic study but not forward-looking breeding processes, which always try to omit inferior genotypes and add more to test performance (KRISHNAMURTHY *et al.*, 2017).

Convenient identification of ideal genotypes and environments is one of the benefits of graphical presentation of GEI. In breeding programs, selection of high-performance genotypes with high stability over years and environment is a great importance. In GGE biplot analysis, an ideal genotype is defined base on its performance and stability. However, the ideal genotype may not exist in reality, and it is a just virtual concept but it can be used as a reference for new

cultivar development. Since PC1 and PC2 units are the original units for yield in the genotype-focused scaling, the units of AEC abscissa (mean yield) and ordinate (stability) should also be the original unit of yield. In turn, the unit of the distance between genotypes and the ideal genotype is the original unit of yield. Consequently, the ranking based on the genotype-focused scaling undertakes that importance of stability and mean yield is the same (YAN and RAJCAN, 2002). Figure 3a indicates that the G5 genotype, which fell into the center of concentric circles, was an ideal genotype in terms of high performance and stability, in comparison to other genotypes.

In the multi-environment experiment, environments tests should consider discrimination ability to discriminate genotypes differences and representativeness ability to represent target region (XU *et al.*, 2014). If one experimental site is similar to another one, then to delete one of the two experimental sites did not affect selecting genotypes when the test cost was less (YAN *et al.*, 2007). Several authors have a lay emphasis on removing of similar environments according to GGE biplot analysis, however the decision on removing an environment or keeping it is very complicated (RAKSHIT *et al.*, 2012; ZHANG *et al.*, 2016; TARIKU, 2017). Non-genetic variations consisted of predictable and non-predictable components. According to LIN and BINNS (1988), soil and weather are the two main elements of an environment or location affecting genotypes performance. Moreover, soil element is normally persistent and can be considered as a fixed parameter. On the other hand, the weather has a predictable component, represented by the general climatic region, and unpredictable component contributed by year-to-year variation (LIN and BINNS, 1988). While developing cultivars with specific adaptation to predictable part of environments (to encounter with a genotype location interaction), plant breeders should also aim to develop cultivars that are adopted to survive unpredictable transient environment variation. Generally, breeding programs take 10-15 years, and stability analysis just takes 2 or 3 years of this period. Therefore, environment evaluation by GGE biplot analysis will be useful when breeders are testing same genotypes and are involved in predictable components of an environment. Overall, it can be concluded that there is a need to test the same set of genotypes across different environments for a long period of time to make this information applicable in future breeding programs. In GGE biplot analysis, the ideal test environment should have large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect) and small (absolute) PC2 scores (more representative of the overall environments). From the results, according to discriminate ability and representativeness, the E4 and E12 environments were found to be perfect environments (Fig. 3b). There were close correlations between E4, E12, E2, E9, E11 and E5 environments, suggesting that indirect selection for better forage yield under one of these environments may be effective to identify the best performing genotypes (Fig. 3b) (TOLESSA *et al.*, 2013).

CONCLUSION

The result of this study indicated that forage yield of foxtail millet was highly influenced by environment and interaction effects. The genotypes exhibited both crossover and non-crossover types of interaction suggesting that selection based on yield can't depict the actual potential of genotypes in breeding programs. Presence of crossover interaction resulted in grouping all the environments into two mega-environments as G5 and G3 genotypes were the best performing in the first and second mega-environments, respectively. Furthermore, six

environments out of 12 environments were highly correlated suggesting that to conduct the MET effectively with limited resources indirect selection for better forage yield under distant environments may be effective to identify the best performing genotypes. The millet breeders in other regions need to identify mega-environments (and correlated environments) and then allocate testing sites accordingly.

Based on results of this study G5 had both high forage yield and good stability. Performances of G5 was better in most locations suggesting that this genotype may be suited for popularization and production in large scale.

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MULTI-LOKACIJSKA PROCENA LINIJA ITALIJANSKOG PROSA ZA STABILNOST PRINOSA KRME

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Izvod

Italijanski proso (*Setaria italica* L. P. Beauv.) je veoma važna biljna vrsta u sušnim i polusušnim područjima. U ovoj studiji testirano je pet poboljšanih linija i jedan komercijalni standard (Bastan) tokom dve godine na šest lokacija. Cilj je bio identifikacija superiornih linija sa naglaskom na visok prinos krme i stabilnost prinosa tokom ispitivanja u multi-lokacijskim ogledima korišćenjem GGE biplot analize. Studija je sprovedena pomoću randomiziranog kompletnog blok dizajna sa četiri ponavljanja. Kombinovana analiza varijanse za prinos krme pokazala je da su genotipovi, lokacije i efekti interakcije veoma značajni ($P \leq 0,01$). Efekti lokacija, genotipa i interakcije činili su 76,38%, 6,97% i 8,92% ukupne varijanse prinosa krme. GGE analiza biplota pokazala je da G5 ima i visoki prinos krme i stabilnost u ispitivanim lokalitetima, a E3 i E4 su bili visoko prinostni u ovom istraživanju. "Ko je najbolji gde" studija je podelila lokacije za testiranje u dva mega-okruženja sa G5 i G3 kao pobjedničkim genotipovima u prvom i drugom mega-okruženju. Prema diskriminiranoj sposobnosti i reprezentativnosti, E4 i E12 bili su savršeni lokaliteti. Generalno, postojanje opsežne unakrsne interakcije jasno je sugerisalo da je potrebno da je veoma značajno da se identifikuju genotipovi specifični za određenu lokaciju tokom multi-lokacijskih ispitivanja u stvaranju novih sorti.

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