

## Evaluation of Genetic Diversity of foxtail Millet (*Setaria italica* (L.) P. Beauv.) Germplasm Based on Different Agronomic Traits

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### ABSTRACT

The present study aimed to investigate the genetic diversity of foxtail millet germplasm and select its superior genotypes. To this end, 130 foxtail millets were studied under an augmented complete block design at Shahid Bahonar University Research Farm. Analysis of Variance (ANOVA) was also carried out on the genotypes to determine the homogeneity of experimental field. The results proved that there were significant differences among the control genotypes regarding all their traits except for the plant loading trait. Based on the results of stepwise regression for grain yield as a dependent variable in foxtail millet genotypes, the number of grains per panicle, the number of panicles, 1000-seed weight, and height respectively had the best efficiency as index traits in selecting genotypes. Based on the cluster analysis, the foxtail millet genotypes were classified into 5 groups. Comparison of the genotypes demonstrated that the genotypes S27 and S7 and cultivar Boston respectively, had the highest biological yield and Bastan and genotype S27 had the highest grain yield among others. The current study revealed that genotypes S7 and S27 had a higher yield than Iran's only breed cultivar, namely Bastan and therefore they can be used in breeding studies in order to produce superior cultivars.

**Keywords:** Biological yield, Cluster analysis, Foxtail millet, Grain yield, Stepwise regression

### INTRODUCTION

Foxtail millet is widely planted in northern China and India. The amount of nutrients this plant contains is as much as or even more than that of wheat or rice. Every single grain has in it 12.3% of protein, 4.7% of fat and 60.6% of carbohydrate. The protein in this grain is as much as that of wheat, but more than that of rice. The amount of minerals, vitamins and amino acid it contains is very high. It also has a high rate of dietary fibers and non-starchy polysaccharide. Furthermore, it has a low glycemic index and releases sugars slowly [1-3]. Foxtail millet is a diploid ( $2n=2x=18$ ) and self-pollinating plant belonging to *Setaria italica*, which is mainly planted in India, Russia, China and Middle Eastern countries [4]. Foxtail millet has so far been under-researched but identifying its genotypes, particularly local genotypes and analyzing its genetic diversity and relations have a key role in improving its yield and in protecting and using this plant [3]. Regarding the low amount of genetic reserves and considering the fact that many useful genes have been lost and also exposition of crops to unhealthy environmental conditions and biotic and abiotic stress, has made studying and identifying genetic diversity in plants a priority which is regarded as the first and foremost step to identifying and maintaining genetic resources which in turn are the basis for carrying out genetic researches and plant breeding programs.

Breeding and producing new cultivars are dependent upon the exact selection among plants which is itself dependent on identifying cultivars and their diversity [5,6]. The most important criterion in identifying superior genotypes is studying morphological markers and yield components. One of the ways to identify the morphological traits relating to yield is identifying the correlation between the traits and the yield [7,8]. Research findings prove that there is positive correlation between grain yield and number of panicles [9]. In their study on 20 foxtail millet genotypes, Channappagoudar et al. [10] demonstrated that there is a significantly positive correlation between grain yield and biomass and plant height, photosynthesis rate and number of

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tillers.

The current research aimed to investigate the genetic diversity in 130 foxtail millet genotypes. It also tried to analyze the relations between morphological traits and grain yield. Clustering the genotypes according to different traits using cluster analysis and selecting superior genotypes was among the final purposes of the present study.

**MATERIALS AND METHODOLOGY**

In the present study, 130 Foxtail millet genotypes (Table 1) were collected from different parts of Iran. They were then planted under an augmented complete block design at Shahid Bahonar University of Kerman Research Farm at a proper time and in the same manner. Each one of the genotypes was planted in a 2 m long line. The distance

between the lines was 40 cm and each plant was at a distance of 8 cm to the next. The processes of planting, growing and harvesting were the common ways as regards those of the foxtail millet. Ten plants were randomly chosen from each plot to measure morphological traits, namely the plant height (cm), panicle length (cm), number of panicles per plant, number of grains per panicle, dry weight per plant (g), grain weight per plant (g), 1000-seed weight, length and width of the leaf (cm), number of tillers per plant, number of leaves, plant loading, bristle color, grain color, grain yield (kg/ha), biological yield (kg/ha) and harvest index. Due to the qualitative nature of the traits like bristle color and grain color, the data were codified in the following way:

Green bristle (1), purple bristle (2), yellow grain (1), brown grain (2), red grain (3).

**Table 1.** Origin of foxtail millet genotypes used in this study.

| Code | Origin        | Code | Origin        | Code | Origin   | Code | Origin  | Code | Origin       | Code | Origin      |
|------|---------------|------|---------------|------|----------|------|---------|------|--------------|------|-------------|
| S1   | -             | S23  | Tabas-5       | S48  | Tabriz-1 | S75  | -       | S99  | Mazanderan-3 | S122 | Esfahan-5   |
| S2   | -             | S24  | -             | S50  | Tabriz-6 | S76  | Gilan-1 | S100 | Yazd-1       | S123 | Esfahan-1   |
| S3   | Shahreko rd-1 | S25  | -             | S52  | Tabriz-2 | S77  | -       | S101 | -            | S124 | -           |
| S4   | Mashhad-4     | S26  | -             | S53  | Tabriz-3 | S78  | -       | S102 | -            | S125 | -           |
| S5   | -             | S27  | -             | S54  | -        | S79  | Gilan-4 | S103 | -            | S126 | Esfahan-2   |
| S6   | Shiraz-1      | S28  | -             | S55  | Tabriz-4 | S80  | Gilan-2 | S104 | Yazd-2       | S127 | Esfahan-3   |
| S7   | -             | S29  | Tabas-6       | S56  | -        | S81  | Gilan-3 | S105 | Yazd-3       | S128 | Esfahan-6   |
| S8   | -             | S30  | Shahreko rd-2 | S57  | -        | S83  | -       | S106 | -            | S130 | Esfahan-7   |
| S9   | Zabol-5       | S31  | Shahreko rd-3 | S58  | -        | S84  | -       | S107 | Yazd-4       | S131 | Esfahan-4   |
| S10  | Yazd-5        | S32  | -             | S60  | Shiraz-2 | S85  | -       | S108 | Kerman-1     | S132 | Khozestan-1 |
| S11  | Kerman-3      | S33  | Shahreko rd-4 | S61  | Shiraz-4 | S86  | -       | S109 | Kerman-2     | S133 | Khozestan-5 |
| S12  | Kerman-4      | S35  | -             | S62  | -        | S87  | Zabol-1 | S110 | -            | S134 | -           |
| S13  | -             | S36  | Mashhad       | S63  | -        | S88  | Zabol-4 | S112 | Kerman-7     | S135 | Khozestan   |

|     |           |     |               |     |              |     |                  |      |          |           |                 |
|-----|-----------|-----|---------------|-----|--------------|-----|------------------|------|----------|-----------|-----------------|
|     |           |     | -1            |     |              |     |                  |      |          |           | -6              |
| S14 | -         | S37 | -             | S65 | -            | S89 | -                | S113 | -        | S136      | Khozestan<br>-2 |
| S15 | Birjand-1 | S38 | Mashhad<br>-2 | S66 | Shiraz-<br>3 | S90 | Zabol-2          | S114 | Kerman-5 | S137      | -               |
| S16 | Birjand-4 | S39 | Mashhad<br>-3 | S67 | Shiraz-<br>5 | S92 | -                | S115 | Kerman-6 | S138      | -               |
| S17 | Birjand-2 | S41 | Mashhad<br>-5 | S68 | Ilam-1       | S93 | -                | S116 | -        | S139      | Khozestan<br>-3 |
| S18 | Birjand-3 | S43 | Mashhad<br>-6 | S69 | -            | S94 | Zabol-3          | S117 | -        | S140      | Khozestan<br>-4 |
| S19 | Tabas-1   | S44 | -             | S70 | Ilam-2       | S95 | -                | S118 | -        | Ss10<br>9 | -               |
| S20 | Tabas-3   | S45 | -             | S72 | -            | S96 | Mazande<br>ran-1 | S119 | -        | C2        | Bastan          |
| S21 | Tabas-2   | S46 | -             | S73 | Ilam-3       | S97 | Mazande<br>ran-2 | S120 | -        |           |                 |
| S22 | Tabas-4   | S47 | -             | S74 | -            | S98 | -                | S121 | -        |           |                 |

To perform variance analysis for the measured traits, based on the type of the augment, randomized complete block design (CRD) was used for control cultivars and the experimental error in this analysis was applied as standard error in different comparisons. To compare the means, LSD was calculated at 1% level and the statistical analysis of the data was carried out according to descriptive statistics like minimum, maximum, mean, standard deviation, diversity coefficient of the traits. Phenotype correlation of the traits and their significance was also measured. To identify the influence of each trait on the yield, linear stepwise regression was used for the grain yield as the dependent variable and other traits as independent variables. Cluster analysis was also carried out applying a Euclidean distance matrix through UPGMA. Statistical software like SAS 9.2, Microsoft Excel 2017, XIStat 2017 were used in this study.

**RESULTS AND DISCUSSION**

In the present research, analysis of the control genotypes was performed according to the randomized complete block design and the results are as presented in **Tables 2 and 3**.

Regarding the implementation of the augment design and the results of the variance analysis, the amount of each of the traits in the genotypes under study was corrected based on the control genotypes. The results showed a significant difference among the control genotypes regarding all the traits except for plant loading. Among the different traits, plant height and leaf length were significant at 5% level. The maximum height was related to genotype S69 (130.60 cm) and the minimum height belonged to genotype S83 (31.60 cm). The longest leaf belonged to genotype S72 (42.30 cm) and shortest was related to genotype S41 (12.30 cm). The traits number of tillers, number of leaves, width of the leaf, length of the panicle, number of the panicles, number of grains per panicle, bristle color, grain color, dry weight per plant, grain weight per plant, 1000-seed weight, harvest index, grain yield and biological yield were also at 1% level of significance. The findings of the research showed that genotypes S27, S7 and Bastan cultivar had the highest biological yield, respectively. Bastan cultivar and genotype S27 also had the highest grain yield among other genotypes.

**Table 2.** ANOVA of different traits in foxtail millet genotypes.

| Source of variation | Df | MS           |                           |                  |            |                     |                |                      |                       |
|---------------------|----|--------------|---------------------------|------------------|------------|---------------------|----------------|----------------------|-----------------------|
|                     |    | Plant height | Number of fertile tillers | Number of leaves | Leaf width | Leaf length         | Panicle length | Dry weight per plant | Seed weight per plant |
| Block               | 3  | 6896.64**    | 0.33 <sup>ns</sup>        | 18.90*           | 0.65**     | 28.92 <sup>ns</sup> | 58.68**        | 118.27**             | 29.73**               |
| Control             | 13 | 365.13*      | 4.10**                    | 50.61**          | 0.45**     | 81.01*              | 74.52**        | 85.99**              | 24.95**               |
| Error               | 39 | 139.11       | 0.23                      | 6.18             | 0.10       | 36.08               | 9.05           | 11.32                | 2.99                  |
| Cv                  | -  | 11.64        | 26.90                     | 22.61            | 16.27      | 23.19               | 18.25          | 29.80                | 29.99                 |

\*\*\* and <sup>ns</sup>: significant at 0.05, 0.01 probability levels and no-significant, respectively

**Table 3.** ANOVA of different traits in foxtail millet genotypes.

| Source of variation | Df | MS                 |                    |                      |                              |                              |              |                  |               |                    |
|---------------------|----|--------------------|--------------------|----------------------|------------------------------|------------------------------|--------------|------------------|---------------|--------------------|
|                     |    | Grain color        | Bristle color      | Plant loading        | Number of grains per panicle | Number of panicles per plant | Grain yield  | Biological yield | Harvest index | 1000-seed weight   |
| Block               | 3  | 0.11 <sup>ns</sup> | 0.16 <sup>ns</sup> | 0.017*               | 211305.26 <sup>ns</sup>      | 0.119 <sup>ns</sup>          | 531308.62**  | 64278082.1**     | 99.01**       | 0.14 <sup>ns</sup> |
| Control             | 13 | 0.67**             | 0.71**             | 0.0039 <sup>ns</sup> | 1138042.75**                 | 1.80**                       | 1204846.07** | 33248180.9**     | 46.99**       | 0.51**             |
| Error               | 39 | 0.08               | 0.07               | 0.004                | 106835.06                    | 0.15                         | 112844.10    | 4870477.1        | 11.65         | 0.10               |
| Cv                  | -  | 22.08              | 16.04              | 9.19                 | 27.60                        | 25.84                        | 29.16        | 28.45            |               | 9.75               |

\*\*\* and <sup>ns</sup>: significant at 0.05, 0.01 probability levels and no-significant, respectively

Based on the corrected traits, the descriptive statistics including the maximum, minimum, mean, standard deviation, diversity coefficient of the traits was calculated. As presented in **Table 3**, the traits number of grains per panicle, biological yield, grain weight per plant, Dry weight per plant and grain yield had the highest diversity coefficient and the 1000-seed weight the lowest. On the whole, most of the traits had high phenotype diversity. The range of variation revealed a wide spectrum for most of the traits which indicated a high diversity among the genotypes under study. As an instance, grain yield range of variation was between 340.63 and 2931.04 and 1769.04 to 29408.45 kg/ha

for biological yield. The high range of variation for most of the traits under study demonstrates a significant variation among Iranian millets. Therefore, selecting a special genotype in Foxtail millet germplasm in order to reach different breeding goals, including improving grain yield and biological yield can be of many benefits [10]. In a similar way observed a significant deviation among these traits in their study on different millets. In order to better investigate the genotypes regarding the traits under question, a comparison of means was made between the genotypes using an LSD method as shown in **Table 4**.

**Table 4.** Descriptive statistics indexes for evaluated different traits in foxtail millet.

|                              | Mean    | Standard deviation | Min     | Max      | %PVC  | LSD1    | LSD2    |
|------------------------------|---------|--------------------|---------|----------|-------|---------|---------|
| Plant height (cm)            | 89.19   | 23.90              | 31.60   | 130.60   | 26.80 | 22.55   | 52.01   |
| Number of fertile tillers    | 2.61    | 1.34               | 1.00    | 7.00     | 51.58 | 0.92    | 2.12    |
| Number of leaves             | 13.17   | 5.12               | 4.36    | 21.85    | 38.88 | 4.75    | 10.97   |
| Leaf width (cm)              | 1.77    | 0.50               | 0.95    | 3.26     | 28.23 | 0.60    | 1.40    |
| Leaf length (cm)             | 28.45   | 7.30               | 12.30   | 42.30    | 25.67 | 11.48   | 26.49   |
| Panicle length (cm)          | 15.87   | 5.70               | 5.57    | 29.46    | 35.95 | 5.79    | 13.37   |
| Number of panicles per plant | 1.95    | 0.99               | 1.00    | 5.00     | 50.92 | 0.75    | 1.75    |
| Dry weight per plant (g)     | 12.99   | 7.10               | 3.22    | 32.95    | 54.67 | 6.43    | 14.84   |
| Seed weight per plant (g)    | 5.62    | 2.93               | 1.85    | 14.44    | 52.23 | 3.31    | 7.63    |
| 1000-seed weight (g)         | 2.77    | 0.56               | 1.20    | 4.26     | 20.42 | 0.62    | 1.43    |
| Grain color*                 | 1.50    | 0.51               | 1.00    | 3.00     | 34.47 | -       | -       |
| Bristle color*               | 1.56    | 0.49               | 1.00    | 2.00     | 31.67 | -       | -       |
| Plant loading                | 0.048   | 0.09               | 0.00    | 0.50     | -     | -       | -       |
| Number of grains per panicle | 1288.85 | 822.93             | 162.71  | 4582.00  | 63.85 | 624.95  | 1441.46 |
| Grain yield (kg/ha)          | 1286.72 | 657.86             | 340.63  | 2931.04  | 51.12 | 642.29  | 1481.45 |
| Biological yield (kg/ha)     | 7915.00 | 4487.65            | 1769.04 | 29408.45 | 59.69 | 4219.66 | 9732.69 |
| Harvest index                | 18.59   | 9.10               | 4.94    | 53.73    | 48.97 | 0.52    | 15.05   |

\* Due to the qualitative nature of these traits, the data were codified numerically. PVC = phenotypic coefficient variance

LSD1: least significant difference for comparing controls

LSD2: least significant difference for compare genotypes with controls

Phenotype correlation was measured through Pearson correlation coefficient as demonstrated in **Table 5**. The results show that the correlation of grain yield with all traits except for 1000-seed weight and number of tillers was positively significant. Among these traits, number of grains per panicle and biological yield had the highest correlation (80%) with grain yield. Channappagoudar et al. [10] also reported a correlation between grain yield and height and biological yield. Santhakumar [11] observed a positive correlation between height, panicle length, biological yield and grain yield. Grain yield and panicle height and length were reported to have had a significant correlation [12].

The present research showed a significant relation between most of the traits except for the 1000-seed weight and biological yield. The correlation is negative (-0.23) with harvest index, but positive with other traits. The harvest index had a significantly positive correlation with grain

yield, but due to the inverse relationship between biological yields, it had a negative correlation. The harvest index showed the highest negative correlation (-0.34) with the number of leaves. There is also a significantly positive relationship between plant height and leaf length, leaf width, panicle length, number of grains per panicle, grain yield and biological yield. The research also proved a significantly positive correlation between leaf length and height, leaf width, panicle length, number of grains per panicle, grain yield and biological yield. Among the traits under study, the highest correlation was related to the number of tillers and number of panicles (0.86), grain yield with number of grains per panicle (0.80) and grain yield with biological yield (0.80). Based on these findings, it can be concluded that the number of grains per panicle and biological yield are the most important traits in improving grain yield in Foxtail millet. Azari Nasrabad and Mirzaee [13] reported similar results and findings in their studies.

**Table 5.** Result of coefficient of genetic correlation between evaluated traits in foxtail millet genotypes.

|     | 1      | 2      | 3       | 4      | 5      | 6       | 4      | 8     | 9      | 10     | 11      | 12 |
|-----|--------|--------|---------|--------|--------|---------|--------|-------|--------|--------|---------|----|
| X1  | 1      |        |         |        |        |         |        |       |        |        |         |    |
| X2  | -0.14  | 1      |         |        |        |         |        |       |        |        |         |    |
| X3  | 0.14   | 0.69** | 1       |        |        |         |        |       |        |        |         |    |
| X4  | 0.60** | -0.18* | 0.001   | 1      |        |         |        |       |        |        |         |    |
| X5  | 0.50** | -0.15  | 0.02    | 0.60** | 1      |         |        |       |        |        |         |    |
| X6  | 0.59** | -0.13  | 0.04    | 0.60** | 0.52** | 1       |        |       |        |        |         |    |
| X7  | -0.12  | 0.86** | 0.60**  | -0.15  | -0.12  | -0.11   | 1      |       |        |        |         |    |
| X8  | -0.02  | -0.05  | -0.14   | -0.03  | -0.06  | -0.08   | -0.004 | 1     |        |        |         |    |
| X9  | 0.53** | -0.06  | 0.10    | 0.70** | 0.36** | 0.49**  | -0.10  | -0.12 | 1      |        |         |    |
| X10 | 0.55** | 0.16   | 0.24**  | 0.63** | 0.33*  | 0.44**  | 0.22*  | 0.10  | 0.80** | 1      |         |    |
| X11 | 0.54** | 0.30** | 0.46**  | 0.66** | 0.46** | 0.48**  | 0.30** | -0.13 | 0.75** | 0.80** | 1       |    |
| X12 | -0.02  | -0.2*  | -0.34** | 0.004  | -0.2*  | -0.0009 | -0.01  | -0.05 | 0.16   | 0.28** | -0.23** | 1  |

\*\*\*: significant at 0.05, 0.01 probability levels, respectively. Plant height (x1), number of tiller (x2), number of leaves (x3), leaf width (x4), leaf length (x5), Panicle length (x6), number of panicles per plant (x7), 1000-seed weight (x8), number of grains per panicle (x9), Grain yield (x10), biological yield (x11), harvest index (x12)

**Table 6** represents the results of the stepwise regression for grain yield (kg/ha) as a dependent variable and other measured traits as independent variables. Biological yield, harvest index, grain weight per plant and dry weight per plant were controlled and not considered as dependent variables due to their close relationship with grain yield. In the regression model and considering the regression coefficient obtained, the traits with higher coefficients have a more important role in improving the yield. Among the measured traits, four traits got into the regression model including the number of grains per panicle, number of panicles, 1000-seed weight and height. The first trait in the model was the number of grains per panicle which had 64.5 of grain yield variation. The R2 related to the regression model shows that almost 81% of grain yield variance change is contingent upon the traits in the model. Dadashi et al. [14] reported that the number of grains per panicle, the number of

fertile tillers, and 1000-seed weight got into the model and according to R2, these traits were reported to have justified 96% of the changes in the grain yield.

The final model obtained for grain yield is as follows:

$$Y = -1.92 + 0.003X1 + 0.06X2 + 1.33X3 + 0.02X4$$

Y, X1, X2, X3 and X4 are grain yield (kg/ha), number of grains per panicle, number of panicles, 1000-seed weight, and height respectively, which are regarded as the main parameters in grain yield. Therefore, in order to select genotypes with high ratios of yield panicles, number of grains per, these traits ought to be taken into consideration. Generally in cereals, the number of fertile panicle, and the average grain weight are regarded as the major components of the yield [15].

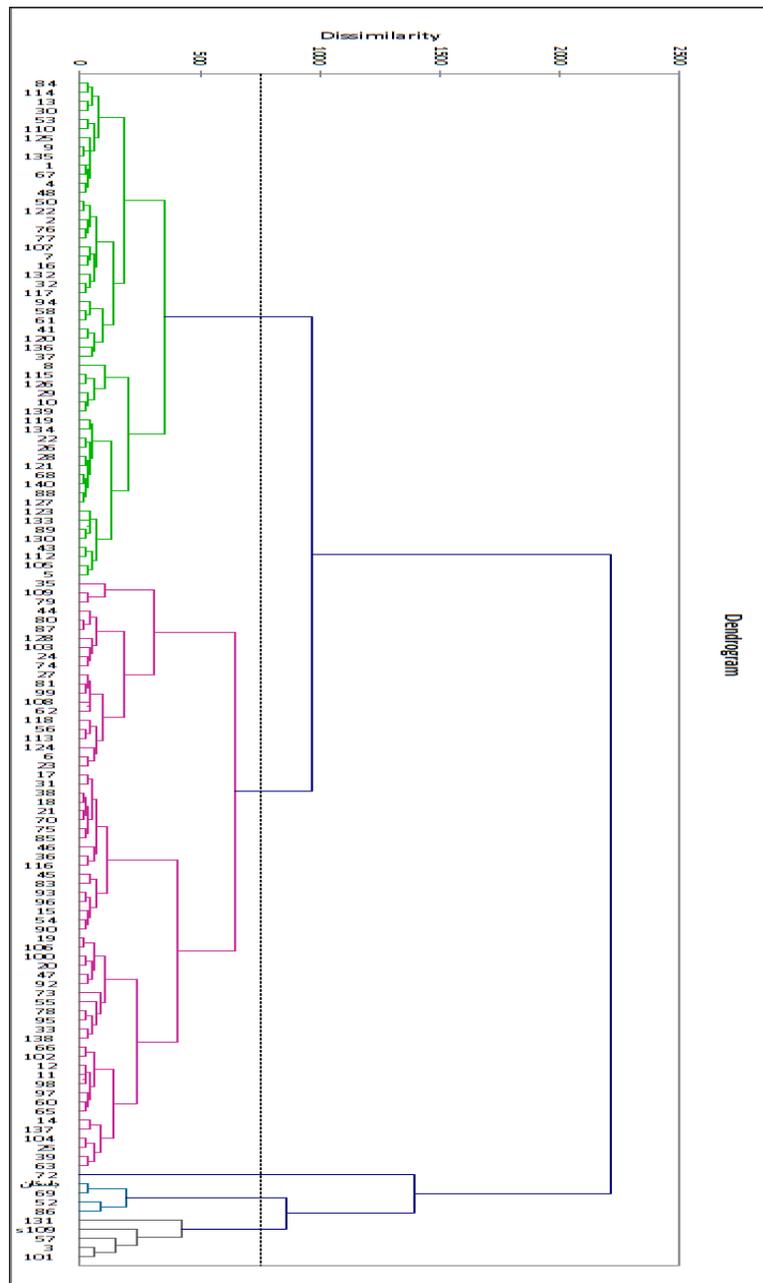
**Table 6.** Results of regression for grain yield in foxtail millet genotypes.

| Fixed variable               | Beta  | Model R-square | Partial R-square | F        |
|------------------------------|-------|----------------|------------------|----------|
| Intercept                    | -1.92 | -              | -                |          |
| Number of grains per panicle | 0.003 | 0.64           | 0.64             | 231.28** |
| Number of panicles per plant | 0.06  | 0.74           | 0.09             | 47.45**  |
| 1000-seed weight             | 1.33  | 0.78           | 0.04             | 25.93**  |
| Plant height                 | 0.02  | 0.81           | 0.03             | 19.50**  |

\*\*\*: significant at 0.05, 0.01 probability levels, respectively

In order to cluster the genotypes under question, cluster analysis was used through UPGMA applying a Euclidean distance matrix. The tree diagram obtained from cluster analysis (**Figure 1**) shows five genotype groups containing 65, 55, 4, 5 and 1 genotypes. The genotypes in group 1 were almost average in most of their traits. Comparison of the characteristics of the genotypes of these groups showed that the mean of the most of the traits related to group 2 genotypes was lower than the total mean and the mean of the traits related to group 3 was higher than that of the total. The genotypes of the group 3 have a great potential regarding their grain yield, biological yield and height. The Bastan

cultivar with high grain and biological yields also belong this group. This is while the genotypes belonging to this group are somehow weak regarding these traits. Therefore, if programs are about to improve grain and biological yields, selecting from group 3 may be a proper choice. Group 4 genotypes are very close to those of group 3 considering most of their traits and had the highest rates regarding 1000-seed weight and harvest index traits. Group 5 contained only 1 genotype, which had a high rate regarding most of the traits. It was reported that cluster analysis using Ward method put the different barley genotypes in 4 groups at normal conditions and 3 at drought stress.



**Figure 1.** Cluster analysis in foxtail millet genotypes with using UPGMA method.

## CONCLUSION

The results of the cluster analysis showed that there is a high genetic diversity in the germplasm under study. Based on the clustering and the estimated means of the traits for the genotypes in each cluster, proper parents can be selected in the improvement programs to carry out purposeful crossings. Since there is closer affinity between the genotypes of these clusters compared to other clusters, crossings between clusters which have more differences can create a higher genetic diversity and heterosis.

To put everything in a nutshell, the present study showed that there was a sufficient diversity in the germplasm considering all the traits investigated. According to the results of the stepwise regression model for grain yield as a dependent variable in Foxtail millet genotypes, the number of grains per panicle, number of panicles, 1000-seed weight, height and number of tillers as indexing traits in selecting genotypes had the highest efficiency in selecting genotypes. Also, the present study revealed genotypes (S27 and S7) that had a higher rate of yield compared to the only Iranian cultivar, namely Bastan. These genotypes can be used in breeding and improving studies to produce superior cultivars.

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