

## Evaluation of Agro-Morphological Diversity in Wild Relatives of Wheat Collected in Iran

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### ABSTRACT

In this study, a core collection of 180 *Aegilops* and *Triticum* accessions belonging to six diploid (*T. boeoticum* Bioss., *T. urartu* Gandilyan., *Ae. speltoides* Tausch., *Ae. tauschii* Coss., *Ae. caudata* L. and *Ae. umbellulata* Zhuk.), five tetraploid (*T. durum*, *Ae. neglecta* Req. ex Bertol., *Ae. cylindrica* Host. and *Ae. crassa* Boiss) and one hexaploid (*T. aestivum* L.) species collected from different regions of Iran were evaluated using 20 agromorphological characters. Statistical analysis showed significant differences among accessions. The Shannon-Weaver ( $H'_{SW}$ ) and Nei's ( $H'_N$ ) genetic diversity indices disclosed intermediate to high diversity for most characters in both *Aegilops* and *Triticum* core sets. In factor analysis, the first five components justified 82.17% of the total of agromorphological variation. Based on measured characters, the 180 accessions were separated into two major groups by cluster analysis. Furthermore, based on the 2D-plot generated using two discriminant functions, different species were separated into six groups, so that distribution of species accorded with their genome construction. Overall, our results revealed considerable levels of genetic diversity among studied Iranian *Aegilops* and *Triticum* accessions, which can open up new avenues for rethinking the connections between wild relatives to explore valuable agronomic traits for the improvement and adaptation of wheat.

**Keywords:** *Aegilops*, Multivariate analysis, Phenotypic diversity, *Triticum*.

### INTRODUCTION

In the recent years, demand for new initial materials has greatly increased due to approaching the limits of biological productivity of wheat. Genetic resources provide opportunities for plant breeders to create novel plant with suitable gene combinations that better matched the conditions of diverse agricultural systems (Glaszmann *et al.*, 2010). Crop Wild Relatives (CWR) are species closely related to crop plants and can contribute useful

traits, such as disease- or pest-resistance and even yield improvement. CWR have a long history of use in wheat breeding, predominantly for pest and disease resistance (Dhaliwal *et al.*, 2002; Zaharieva *et al.*, 2003; Schneider *et al.*, 2008; Hovhannisyan *et al.*, 2011; Petersen *et al.*, 2015), and are expected to be increasingly used in the search for tolerance to biotic and abiotic stresses (Trethowan and Mujeeb-Kazi, 2007; Ali *et al.*, 2013; Kiani *et al.*, 2015; Masoomi-Aladizgeh *et al.*, 2015; Pour-Aboughadareh *et al.*, 2017a).

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The genus *Aegilops* L. and *Triticum* belong to the tribe *Triticeae* within the *Pooideae* subfamily of the grass family *Poaceae*. These genera are important wheat gene pool, because they are evolutionarily related to the major agricultural crop *T. aestivum* L. Kimber and Feldman (1987) and van Slageren (1994) described 22 *Aegilops* and five *Triticum* species, including diploid ( $2n=2x=14$ ), tetraploid ( $2n=4x=28$ ) and hexaploid species, among which diploid species with useful characters are of potential use in wheat breeding (Appels and Lagudah, 1990). Wild relatives of common wheat have been widely distributed in the Middle and Near East. Iran, with a total land area of 1,648,000 square kilometers, lies between  $25^{\circ}$  and  $39^{\circ}$  N latitude and  $44^{\circ}$  and  $63^{\circ}$  E longitude and is primarily temperate in the northern half part, subtropical in the southern half and mostly desert in the middle. The resultant variability in climate and environment has made an extensive diversity of plant germplasm. In the Fertile Crescent, Iran is known as one of the primary centers of distribution of wild relatives of wheat, so that compositions of *Aegilops* and *Triticum* species, as the richest wheat gene pool, have been found in this country. The conditions of the origins of wild relatives of common wheat in different parts of Iran, especially northwest, west and southwest regions (east of the Fertile Crescent), also suggest these areas are one of the ideal regions for discovering suitable genes to be transferred into cultivated modern wheat (Zohary and Hopf, 2000).

The botanical and agro-morphological characters are the first and easiest criteria for the assessment of genetic diversity in natural populations and useful as a guide to follow up characterization and evaluation studies. Many studies showed that agro-morphological characteristics are very useful in identification and evaluation of genetic diversity in wild wheat germplasm as well as having the advantage of providing direct tools in the field for assessing plant performance and select suitable varieties (Salimi et al., 2005; Naghavi et al., 2009a;

Kiani et al., 2015; Zhang et al., 2015; Alsaleh et al., 2016). In the study conducted by Ehdaie and Waines (1989), moderate to high heritability estimates for phenological and agro-morphological characters in core collection of native wheat landraces collected in southwestern Iran were reported. In another study, Moghaddam et al. (1997) reported great values of phenotypic and genotypic coefficient of variability as well as genetic advance for number of tillers, number of spikes, 1000-seed weight and grain yield per plant of Iranian wheat landraces, indicating that selection for these characters might be effective for wheat improvement. Zaharieva et al. (2003), analyzing several populations of *Ae. geniculata*, *Ae. neglecta* and *Ae. cylindrica* sampled in Bulgaria, indicated significant differences between populations for most of the morphological characters. Arzani et al. (2005) evaluated diversity in wild relatives of wheat collected from different eco-geographical regions of Iran and indicated considerable variation among species for qualitative characters along with significant differences for inter and intraspecific variation for the measured characters.

Description and identification of the genetic diversity available in wheat germplasm are the basis of improved plans designed to control genetic erosion; they are also an initial requirement for the utilization of useful characters in plant breeding. Although several studies using protein and molecular markers have shown a high level of genetic diversity in Iranian wheat germplasm (Moghaddam et al., 2000; Dudnikov and Kawahara, 2006; Naghavi et al., 2007; Naghavi et al., 2009b; Tahernejhad et al., 2010; Ehtemam et al., 2010; Moradkhani et al., 2012; Mousavifard et al., 2015), no systematic work has been undertaken until now to collect, describe, and evaluate native *Aegilops* and *Triticum* germplasm in Iran. To increase the usefulness of exploitation of wild relatives for wheat improvement, the morphological characterization is required. Therefore, a

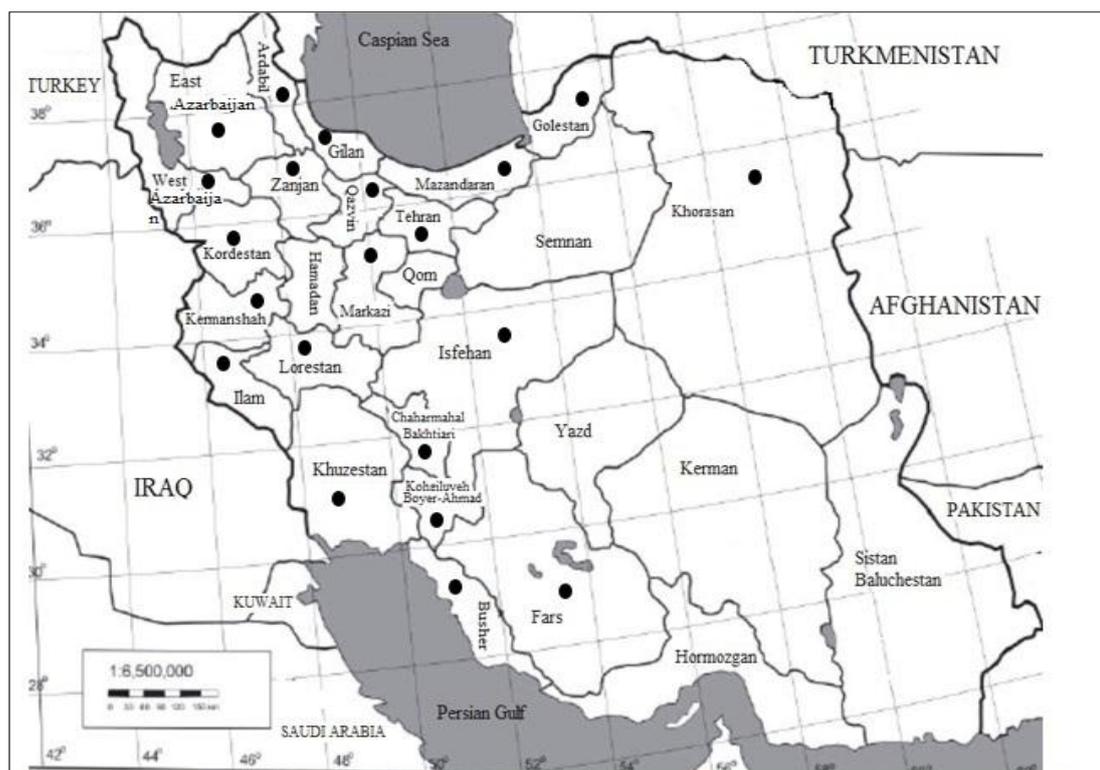
survey of the genetic diversity is necessary to management of breeding programs involving the native wheat germplasm. The current work was undertaken in order to assess the agro-morphological diversity in core collection of wild relatives of wheat collected in different eco-geographical regions of Iran.

## MATERIALS AND METHODS

### Plant Materials

A core collection of 180 *Triticum* and *Aegilops* accessions were sampled in natural habitats, desert, valleys, and mountains from Zagros and central Elburz mountains located in a wide range from north, northwest,

northeast to south and southwest of Iran (Figure 1). The evaluated accessions in this study consisted of: Six diploid- *T. boeoticum* Boiss. ( $A^b$  genome), *T. urartu* Gandilyan. ( $A^u$  genome), *Ae. speltoides* Tausch. (S genome), *Ae. tauschii* Coss. (D genome), *Ae. caudata* L. (C genome) and *Ae. umbellulata* Zhuk. (U genome); Five tetraploid- *T. durum* Def. (AB genome), *Ae. neglecta* L. (UM genome), *Ae. cylindrica* Host (DC genome) and *Ae. crassa* Boiss (DM genome) and *Ae. triuncialis* (CU genome), and One hexaploid- *T. aestivum* L. (ABD genome) species. All accessions were preserved in the Gene Bank of the University of Ilam. Detailed information about genome species and eco-geographical distribution of these materials is listed in supplementary Table 1.



**Figure 1.** Geographical zones of the 180 Iranian *Aegilops* and *Triticum* accessions used in this study (black circles indicate sampling locations).

**Table 1.** Mean, maximum, minimum, Standard Deviation (SD) and coefficient of the variation values for each character evaluated for *Aegilops* and *Triticum* core collection.<sup>a</sup>

| Character         | <i>Aegilops</i> |        |        |       |       |        | <i>Triticum</i> |        |        |        |       |       |        |           |
|-------------------|-----------------|--------|--------|-------|-------|--------|-----------------|--------|--------|--------|-------|-------|--------|-----------|
|                   | Mean            | Min    | Max    | SD    | CV    | $H'_N$ | $H'_{SW}$       | Mean   | Min    | Max    | SD    | CV    | $H'_N$ | $H'_{SW}$ |
| DH (Day)*         | 170.53          | 152.75 | 186.75 | 6.40  | 3.75  | 0.50   | 0.49            | 165.80 | 149.37 | 188.62 | 9.18  | 5.54  | 0.61   | 0.82      |
| DB (Day)          | 162.94          | 148.50 | 180.50 | 4.59  | 2.82  | 0.41   | 0.56            | 157.93 | 139.63 | 170.38 | 8.08  | 5.11  | 0.63   | 0.75      |
| DA (Day)          | 174.84          | 155.50 | 201.50 | 7.56  | 4.32  | 0.58   | 0.76            | 170.63 | 160.25 | 195.75 | 8.22  | 4.81  | 0.50   | 0.78      |
| DM (Day)          | 202.37          | 185.38 | 209.63 | 5.16  | 2.55  | 0.49   | 0.73            | 195.18 | 176.13 | 212.13 | 7.77  | 3.98  | 0.60   | 0.89      |
| GFP (Day)         | 27.53           | 7.88   | 52.88  | 5.94  | 21.57 | 0.49   | 0.56            | 24.55  | 9.63   | 35.38  | 5.31  | 21.62 | 0.54   | 0.80      |
| PH (cm)           | 25.36           | 11.16  | 52.46  | 7.43  | 29.30 | 0.58   | 0.73            | 71.95  | 43.02  | 118.48 | 14.16 | 19.67 | 0.65   | 0.83      |
| PL (cm)           | 5.27            | 0.85   | 19.45  | 3.47  | 65.81 | 0.42   | 0.53            | 22.08  | 5.61   | 76.63  | 12.47 | 56.50 | 0.23   | 0.34      |
| NN                | 3.26            | 1.78   | 5.03   | 0.65  | 19.99 | 0.57   | 0.55            | 3.41   | 2.98   | 5.03   | 0.54  | 15.81 | 0.47   | 0.66      |
| STD (mm)          | 1.47            | 0.87   | 2.67   | 0.41  | 27.81 | 0.56   | 0.83            | 2.94   | 1.99   | 4.82   | 0.66  | 22.64 | 0.40   | 0.59      |
| LL (cm)           | 4.97            | 1.26   | 12.80  | 2.69  | 54.08 | 0.68   | 0.86            | 11.36  | 3.50   | 24.06  | 6.19  | 54.44 | 0.63   | 0.96      |
| LW (cm)           | 0.47            | 0.18   | 1.00   | 0.19  | 40.15 | 0.41   | 0.97            | 0.85   | 0.31   | 1.99   | 0.41  | 47.65 | 0.42   | 0.61      |
| NFT               | 35.10           | 4.53   | 152.98 | 22.46 | 63.99 | 0.37   | 0.73            | 8.62   | 2.25   | 18.25  | 3.18  | 36.89 | 0.93   | 0.80      |
| SL (cm)           | 6.57            | 1.73   | 11.93  | 3.07  | 46.71 | 0.74   | 0.99            | 9.28   | 4.88   | 13.80  | 1.76  | 18.92 | 0.83   | 0.67      |
| NSp               | 6.08            | 1.63   | 11.63  | 2.30  | 37.78 | 0.71   | 0.94            | 18.48  | 11.73  | 27.07  | 3.33  | 18.00 | 0.91   | 0.89      |
| NGSp              | 2.54            | 1.01   | 5.59   | 0.95  | 37.34 | 0.31   | 0.49            | 1.91   | 0.85   | 2.95   | 0.52  | 27.43 | 0.24   | 0.59      |
| NGS               | 14.78           | 3.51   | 28.51  | 5.83  | 39.43 | 0.70   | 0.92            | 34.62  | 14.30  | 59.90  | 8.87  | 25.62 | 0.65   | 0.85      |
| GW (g)            | 9.98            | 1.06   | 19.09  | 3.20  | 32.04 | 0.49   | 0.66            | 28.03  | 12.12  | 57.38  | 12.74 | 45.44 | 0.62   | 0.83      |
| GY (g per plant)  | 4.75            | 0.02   | 20.52  | 3.37  | 70.94 | 0.46   | 0.57            | 8.01   | 0.33   | 22.08  | 4.64  | 57.89 | 0.71   | 0.95      |
| Bio (g pre plant) | 31.58           | 8.38   | 98.38  | 13.92 | 44.09 | 0.53   | 0.66            | 38.73  | 10.82  | 91.18  | 19.23 | 49.66 | 0.84   | 0.91      |
| HI (%)            | 15.90           | 1.92   | 60.88  | 10.70 | 67.33 | 0.53   | 0.68            | 19.84  | 2.80   | 46.22  | 9.10  | 45.84 | 0.69   | 0.92      |

<sup>a</sup> DH: Days to Heading; DB: Days to Booting; DA: Days to Anthesis; DM: Days to physiological Maturity; GFP: Grain Filling Period; PH: Plant Height; PL: Peduncle Length; LL: Leaf Length; LW: Leaf Width; NN: No. of stem Node; STD: Stem Diameter; NFT: No. of spikes; SL: Spike Length, NSp: No. of Spikelets per spike, NGSp: No. of Grains per Spikelet, NGS: No. of Grains per sSpike, GW: 1000-Grain Weight, GY: Grain Yield per plant; Bio: Biomass yield per plant; HI: Harvest Index; SD: Standard Deviation; CV: Coefficient of Variation;  $H'_N$ : Shannon-Weaver diversity index (Hutchenson, 1970),  $H'_{SW}$ : Nei's ( $H'_N$ ) diversity index (Nei, 1973).

### Experimental Design and Agro-Morphological Characters

The field experiment was conducted in Research Station of the Faculty of Agriculture, University of Ilam, Iran (Latitude 33° 39' N, Longitude 46° 22' E, and Altitude of 1,445 m above sea level) in 2014-2015 cropping seasons, and 20 agro-morphological characters were recorded following International Board for Plant Genetic Resources, 1985. Augmented design with two blocks was used with eight repeated check accessions randomly arranged in each block. The trial consisted of 192 experimental plots, arranged in two blocks, with 96 entries in each block and a different randomization in each location. The plot size was one row 3 m long with 30 cm spacing between rows. The climate is characterized by mean annual precipitation of 244 mm per year; mean annual minimum and maximum temperature of 22.6 and 11°C, respectively, and annual mean temperature of 16.8°C. The agro-morphological characters were: (1) Number of Days to Booting from emergence (DB), (2) To Heading (DH), (3) To Anthesis (DA), (4) Physiological Maturity (DM), (5) Grain Filling Period (GFP), (6) Plant Height (PH), (7) Peduncle Length (PL), (8) Flag Leaf Length (LL), (9) Flag Leaf Width (LW), (10) Number of stem Node (NN), (11) Stem Diameter (STD), (12) Number of Fertile Tillers per plant (NFT), (13) Main Spike Length (SL), (14) Number of Spikelets per spike (NSp), (15) Number of Grains per Spikelet (NGSp), (16) Number of Grains Per Spike (NGS), (17) 1000-Grain Weight (GW), (18) Grain Yield per plant (GY), (19) Biomass yield per plant (Bio), and (20) Harvest Index (HI). The characters 6-17 were measured on the basis of five randomly chosen plants per plot. Grain yield and biomass recorded on a per plot basis were converted to per plant basis. Harvest index was determined from the ratio of grain yield to biomass.

### Statistical Analysis

Augmented design analysis and adjusted means was computed following the SAS PROC GLM (SAS Inc. 2011) outlined by Wolfinger (1997). Descriptive statistics (i.e.: mean, range, variance, standard deviation, and coefficient of variability) for agro-morphological characters were determined for qualitative descriptors. Shannon-Weaver ( $H'_{sw}$ ) and Nei's ( $H'_N$ ) diversity indices and for agro-morphological characters in both *Aegilops* and *Triticum* genus was estimated as follows (Hutchenson, 1970; Nei, 1973):

$$H'_{sw} = \sum_{i=1}^n (p_i \log_e p_i)$$

Where,  $P_i$  and  $n$  are the proportion of accessions in the  $i$ th class of  $n$ -class characters, and the number of phenotypic classes for a given character, respectively. Then, the standardized  $H'_{sw}$  values, which ranged from zero to one, were obtained from ration of  $H'_{sw}$  to  $\log_e$  of total number of phenotypic classes. The diversity index was classified as high ( $H' \geq 0.60$ ), intermediate ( $0.40 \leq H' \leq 0.60$ ) and low ( $0.10 \leq H' \leq 0.40$ ) as described by Eticha *et al.* (2005).

$$H'_N = 1 - \left( \sum_{i=1,2,3,\dots}^n x_i^2 \right)$$

Where,  $X_i$  is the fraction of individuals in the  $i$  class for a given character,  $n$  is the number of classes.

Principal components analysis was used to extract factorial load of matrix and also to estimate the number of factors. Then, the factors which had a root bigger than one were used to form factorial coefficients matrix. By means of varimax rotation, rotation was done on the major factorial loads matrix and the matrix of rotated factorial loads was obtained (Harman, 1976; Poursiahbidi *et al.*, 2012). Cluster analysis were performed using the Hierarchical Cluster Analysis (HCA), and the Euclidean distance was used as a dissimilarity measure required in Ward's clustering method



(Ward, 1963). Also, the discriminant analysis test was used to estimate the optimal number of clusters. Two discriminant functions (Manly, 1994) were calculated using Euclidean distance due to agro-morphological data and then multidimensional scaling plot based on Dis1 and Dis2 were performed for classification of the 180 accessions as well as identification of species of each major genus. The statistical analysis was performed by SPSS version 16.0 software (SPSS Inc. 2007).

## RESULTS

### Morphological Characteristics of *Aegilops* and *Triticum* Core Sets

Mean, minimum, maximum, Standard Division (SD), variance and Coefficient of

Variance (CV) values for 20 agro-morphological characters measured for both *Triticum* and *Aegilops* core sets are given in Table 1. Results indicated a large variation in germplasm accessions based on morphological characters. Large variation among *Triticum* accessions was found for Bio followed by PH, GW, PL, DH, HI and NGS. The highest Coefficient of Variance (CV) was observed for GY, PL, LL, Bio, LW, HI, and GW, respectively. Also, across the studied 109 accessions of *Aegilops* core set, GY, HI, PH, NFT, LL, SL and Bio showed the higher CV value ( $CV \geq 44.09\%$ ) indicating a high level of variation. The remaining characters exhibited low to intermediate CV values, ranging from 2.55 to 39.43% (Table 1). Taking into account of agro-morphological means comparison, we identified the best species for each character (Supplementary Table 2). For instance, the shorter time of development such as DH,

**Table 2.** The first five components extracted in factor analysis for agro-morphological characters in the 180 accessions of *Aegilops* and *Triticum*.

| Characters                           | FA1                     | FA2         | FA3          | FA4         | FA5         |
|--------------------------------------|-------------------------|-------------|--------------|-------------|-------------|
| Days to heading (Day)                | -0.17                   | <b>0.91</b> | 0.01         | -0.15       | -0.04       |
| Days to booting (Day)                | -0.25                   | <b>0.87</b> | -0.11        | -0.05       | -0.03       |
| Days to anthesis (Day)               | -0.17                   | <b>0.84</b> | 0.03         | -0.05       | -0.33       |
| Days to physiological maturity (Day) | -0.13                   | <b>0.87</b> | -0.26        | -0.12       | 0.25        |
| Grain filling period (Day)           | 0.08                    | -0.08       | -0.35        | -0.08       | <b>0.76</b> |
| Plant height (cm)                    | 0.49                    | -0.30       | <b>0.76</b>  | 0.10        | 0.01        |
| Peduncle length (cm)                 | 0.14                    | -0.46       | <b>0.76</b>  | 0.07        | -0.11       |
| Number of node                       | 0.03                    | 0.01        | 0.21         | 0.28        | 0.78        |
| Stem diameter (mm)                   | <b>0.84<sup>a</sup></b> | -0.22       | 0.37         | 0.13        | -0.01       |
| Leaf length (cm)                     | <b>0.86</b>             | -0.21       | 0.13         | 0.16        | 0.21        |
| Leaf width (cm)                      | <b>0.90</b>             | -0.14       | 0.03         | 0.16        | 0.19        |
| Number of fertile tillers            | <b>-0.66</b>            | 0.09        | -0.31        | 0.34        | 0.25        |
| Main spike length (cm)               | <b>0.59</b>             | 0.25        | 0.42         | -0.23       | -0.18       |
| Number of spikelets per spike        | <b>0.53</b>             | -0.06       | <b>0.74</b>  | 0.10        | -0.22       |
| Number of grains per spikelet        | -0.03                   | -0.26       | <b>-0.75</b> | 0.40        | -0.16       |
| Number of grains per spike           | <b>0.75</b>             | -0.21       | 0.37         | 0.33        | -0.17       |
| 1000-Grain weight (g)                | <b>0.75</b>             | -0.25       | 0.21         | 0.36        | 0.15        |
| Grain yield per plant (g)            | 0.42                    | -0.20       | -0.02        | <b>0.78</b> | 0.14        |
| Biomass yield per plant (g)          | 0.49                    | -0.32       | -0.16        | -0.08       | 0.01        |
| Harvest index (%)                    | 0.02                    | -0.07       | -0.03        | <b>0.94</b> | 0.07        |
| Eigenvalue                           | 8.06                    | 3.35        | 2.23         | 1.49        | 1.31        |
| Percentage                           | 40.30                   | 16.73       | 11.15        | 7.45        | 6.54        |
| Cumulative                           | 40.30                   | 57.03       | 68.18        | 75.62       | 82.17       |

<sup>a</sup> Bold values are Eigenvalues  $\geq 0.50$ .

DA, DM, LL and LW belonged to the *T. durum*, *T. aestivum*, *Ae. crassa*, and *T. boeoticum* species, respectively. *T. urartu*, *T. boeoticum*, *Ae. crassa*, and *Ae. umbellulata* indicated the lowest value for GFP. Also, the highest value of PH, PL and NGS were recorded for *T. urartu*, *T. boeoticum*, *T. aestivum*, and *T. urartu* species. In the case of Bio and HI characters, the highest values were obtained for *T. durum*, *T. aestivum*, *Ae. umbellulata*, and *Ae. cylindrica*, respectively.

### Phenotypic Diversity Indices

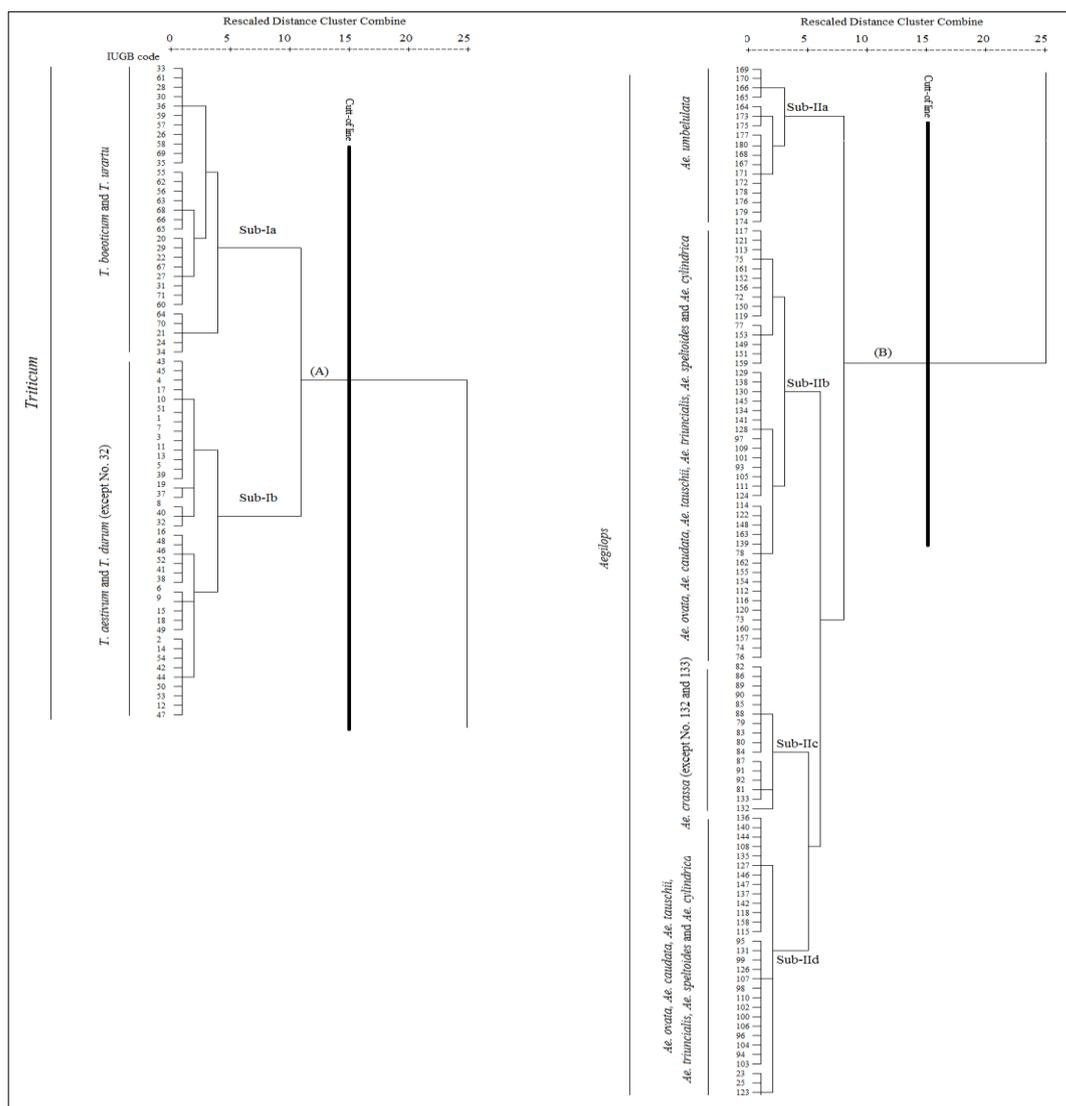
Great diversity was observed for both *Aegilops* and *Triticum* accessions. Speciously, there was high-level variability for all characters analyzed (Table 1). For accessions related to *Aegilops* core set, estimates of Nei's diversity index ( $H'_N$ ) for characters ranged from 0.31 (for NGSp) to 0.74 (for SL) with an overall mean of 0.53. Most characters indicated intermediate level of diversity. However, among the characters the highest value ( $H'_N > 0.60$ ) was observed for SL, NSp, NGS and LL. Shannon-Weaver diversity index ( $H'_{sw}$ ) varied between 0.49 (DH) and 0.99 (SL) with an average of 0.71. Most characters showed relatively high levels of diversity, such that the highest value was estimated for SL followed by NSp, LW, NGS, LL, STD, NFT, PH, DB and DM. In contrast, in the *Triticum* core set, most characters showed high level of diversity ( $H'_N > 0.60$ ).  $H'_N$  values ranged from 0.23 to 0.93 with an average 0.61, and the highest value was observed for NFT, NSp, Bio, SL, GY, HI, PH, NGS, LL, DB, DH, and GW, respectively. The remaining characters exhibited relatively low to intermediate levels of diversity ( $0.54 < H'_N < 0.23$ ). In the case of  $H'_{sw}$ , estimates varied from 0.34 for PL to 0.96 for LL with an overall mean of 0.77. In particular, those with values exceeding 0.80 were LL, GY, HI, Bio, HI, NSp, DM, NGS, GW, PH, DH, and NFT; while those with  $H'_{sw}$  values below 0.60 were NGSp, STD, and PL.

### Factor Analysis

The factor analysis result indicated that five components explained 82.17% of the total variation contributed by characters (Table 2). The first component justified 40.30% of the total variation, which is strongly influenced by STD, LL, LW, NFT, SL, NSp, NGS, and GW. The second component accounted for 16.73% of the total variation and was mainly explained by developmental characters such as DH, DB, DA, and DM. The third component justified 11.15% of the total variation and was only influenced by PH, PL, NSp, and NGSp. The fourth component indicated 7.45% of the total variation and presented GY and HI. Finally, the fifth component accounted for 6.54% of the changes that were explained by other four previous components including GFP.

### Cluster Analysis and Scoter Plot

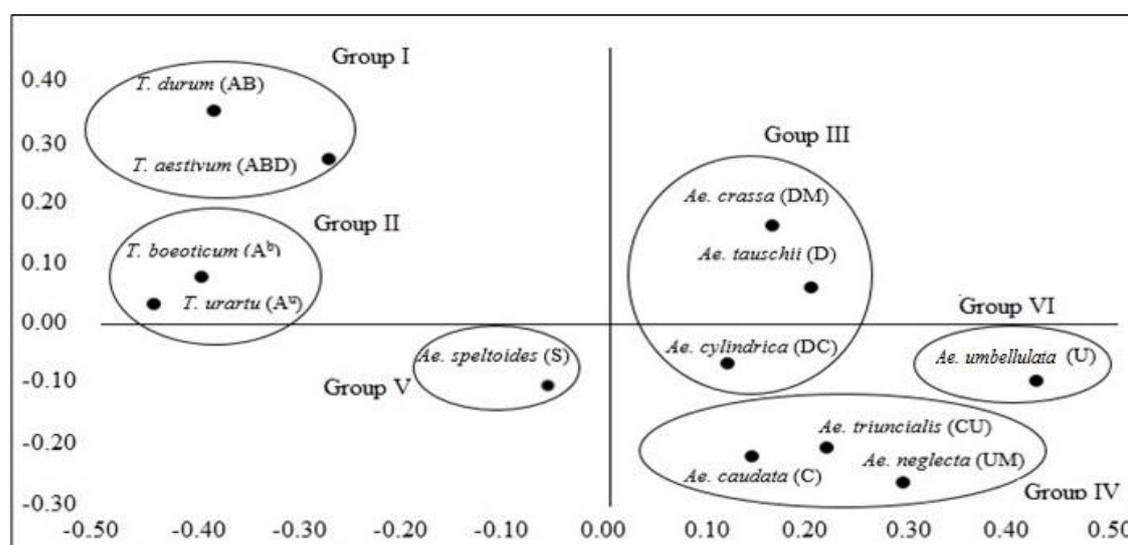
Ward's cluster analysis using Euclidean distance coefficient was used in order to divide the individuals into few clear clusters. According to the results of the discriminant analysis test, the dendrogram obtained from cluster analysis separated all accessions into two main clusters at rescaled distance level of 15 (Figure 2). The first main cluster (A) was divided into two sub-clusters (Ia and Ib). The accessions related to *Triticum* set, *T. aestivum* (accession number 1-19), *T. boeoticum* (accession number 20-36), *T. durum* (accession number 37-54), and *T. urartu* (accession number 55-71), were distinguished in the first main cluster. Einkorn wild wheats, *T. urartu* and *T. boeoticum*, classified as sub-cluster Ia. Sub-cluster Ib included all accessions of *T. aestivum* and *T. durum* along with one accessions of *T. boeoticum*. The second main cluster (B) was divided into four sub-clusters (IIa, IIb, IIc and IId) and comprised all accessions of *Aegilops* set, *Ae. caudata* (accession number 72-78), *Ae. crassa* (accession number 79-92), *Ae. cylindrica*



**Figure 2.** Dendrogram of 180 accessions of Iranian *Aegilops* and *Triticum* constructed for 20 agromorphological characters using Ward's method based on Euclidean distance.

(accession number 93-111), *Ae. neglecta* (accession number 112-122), *Ae. speltoides* (accession number 123-128), *Ae. tauschii* (accession number 129-148), *Ae. triuncialis* (accession number 149-163), and *Ae. umbellulata* (accession number 164-180) as well as two accessions of *T. boeoticum*. The accessions of *Ae. umbellulata* were placed in the sub-cluster IIa. Sub-cluster IIb included 46 accessions with several groups. All accessions of *Ae. caudata* and several accessions of *Ae. neglecta*, *Ae. cylindrica*, *Ae. tauschii*, *Ae. triuncialis* and *Ae.*

*speltoides* were distributed into this sub-cluster. *Ae. crassa* accessions separately clustered in the third sub-cluster (Iic), moreover, two accessions of *Ae. tauschii* existed in this sub-cluster. Finally, the fourth sub-cluster (Iid) consisted of other accessions of *Aegilops* set as well as two accessions of *T. boeoticum*. In addition, multidimensional analysis clustered different species into six groups. As shown in 2D-plot (Figure 3), *T. aestivum* and *T. durum* species grouped in distinct group (I). *T. boeoticum* and *T. urartu* were placed in group II. Group



**Figure 3.** The multidimensional scaling plot of species form of Iranian *Aegilops-Triticum* core collection using Euclidean distance coefficient.

III included *Ae. tauschii*, *Ae. crassa* and *Ae. cylindrica* species. *Ae. caudata*, *Ae. neglecta*, and *Ae. triuncialis* were clustered in the same group (IV). Finally, *Ae. speltoides* and *Ae. umbellulata* separately formed two groups (V and VI).

## DISCUSSION

Description of agro-morphological characters is an important requirement for effective and efficient utilization of wild relatives of wheat in breeding programs. In the current work, 180 wild wheat accessions belonging to 12 different species were evaluated with 20 phenological and agro-morphological characters (Table 1) and a comprehensive statistical analysis was performed. According to the results, there was a high level of variability for all agro-morphological characters among *Aegilops* and *Triticum* core sets sampled in different eco-geographical regions of Iran. In the case of both *Aegilops* and *Triticum* sets, most characters such as PH, PL, LL, LW, NFT, SL, NSp, NGS, GW, GY, Bio, and HI were highly variable between accessions, for which the CV was, > 20% (Table 1). The

variation exhibited by these accessions in developmental and quantitative characters indicates that selection for several of these characters might be effective (Moghaddam *et al.*, 1997). In the case of developmental characters, there was no significant difference between *Aegilops* and *Triticum* accessions. However, the result showed that the accessions from *Triticum* set tend to be late in heading, anthesis, and physiological maturity as compared to *Aegilops* accessions. In the present study, *T. durum*, *T. aestivum*, *Ae. crassa*, and *T. boeoticum* species were found to be early heading and to have shorter grain filling period ranging from 32 to 37 days (Table 2). In Mediterranean dry area as well as similar origins, early heading and early maturity types are suitable because they provide the especial conditions for plants to escape the terminal drought stress that usually occurs in these regions (Rawashdeh *et al.*, 2007). The length of peduncle is coincident with plant height. Borner *et al.* (1996) noted plant height and peduncle length are important in disease escape and breeding for resistance to head diseases, because plants with shorter height and length of peduncles are more susceptible. In the case of these characters,



accessions of *T. boeoticum* and *T. urartu* had the highest values of PH and PL. In this study, high variation in the NFT and NGS were found which, provide the opportunity for breeders to select accessions with high number of spikes and grains per spike (Table 2). This also has been found by Pathak and Nema (1985) in India, who reported that selection for these traits might be effective to improve the landraces of Indian wheat. However, it should be mentioned that increasing tillering capacity may be an undesirable character in dry climates, because it might result in fast soil moisture depletion, which may be required later in the season, at the critical stages of crop development (Hurd, 1971). Also, different levels of variation were observed for grain yield and its related characters, biomass, and harvest index in the present study. Among the different species of *Aegilops*, *Ae. crassa*, *Ae. cylindrica*, and *Ae. umbellulata* similar to *T. aestivum* and *T. durum* represent considerable potential for GW, GY, Bio, and HI characters. Hence, these species can be attractive for breeding programs (Table 2).

The Shannon-Weaver ( $H'_{SW}$ ) and Nei's ( $H'_N$ ) indices were selected (Table 1) as two best parameters to assess phenotypic diversity within studied accessions. The values of the  $H'_{SW}$  and  $H'_N$  revealed considerable level of phenotypic diversity within these germplasm. Overall, in both *Aegilops* and *Triticum* accessions core sets, all selected quantitative characters showed intermediate to high diversity using  $H'_{SW}$  and  $H'_N$  indices, suggesting that the Iranian wild relatives of wheat could be considered as an ideal natural source of agro-morphological variation. Thus, detection of their genetic variability is very useful for future breeding programs. Similarly, phenotypic variability among wheat germplasms from Iran was previously reported (Moghaddam et al., 1997; Salimi et al., 2005; Arzani et al., 2005; Aghaei et al., 2008; Pour-Aboughadareh et al., 2017b).

In the present study, the factor analysis showed that 82.17% of the total variation explained for five components axis for 20

quantitative characters (Table 2). The variance accumulated by the first two components was 57.03%, in which agro-morphological characters had relatively high percentage of the total variation, which, according to Mardia et al. (1979), explains acceptably the variability revealed between individuals. As shown in dendrogram obtained by cluster analysis, the 180 accessions separated into two major groups, so, some inter and intra-species dissimilarities observed in each group represent intermediate agro-morphological variation between studied accessions (Figure 2). Multidimensional scaling analysis revealed clear pattern of calcification and distributions of studied accessions, such that this analysis not only confirmed the results obtained from cluster analysis, but also indicated extreme variation among accessions and their grouping. Moreover, as shown in 2D-plot (Figure 3), different species were recognized based on genome construction. All investigated species were relatively well clustered. *T. aestivum* and *T. durum* species, respectively with ABD and AB genomes, grouped in a distinct group (I). *T. boeoticum* and *T. urartu*, as A-genome donor to common and durum wheats, were placed in group II. The species possessing D-genome such as *Ae. tauschii* (D), *Ae. crassa* (DM) and *Ae. cylindrica* (DC) formed the third group (III). The fourth group comprised of three close species, namely, *Ae. caudata* (C), *Ae. neglecta* (MU), and *Ae. triuncialis* (CU). *Ae. umbellulata* and *Ae. speltoides* with U and S genomic constitution individually placed in separately two groups (V and VI). In general, based on abovementioned results, it can be suggested that there are different sub-gene pools within Iranian *Aegilops-Triticum* germplasm. Similarly, Arzani et al. (2005), Ranjbar et al. (2007), Aghaei et al. (2008), and Tahernezhad et al. (2010), using agro-morphological characters, found high levels of genetic diversity in the wild relatives of wheat accessions collected from different eco-geographical of Iran.

## CONCLUSIONS

Rich germplasm of *Aegilops* and *Triticum* has been reported from Iran (Skovmand *et al.* 2002), but little information is accessible about its distribution and diversity. Skovmand *et al.* (2002) also reported that in the Iranian mountains and deserts, especially Zagros Mountains, there are natural genetic resources of abiotic stress such as salt-tolerance and drought-tolerance. Agromorphological genetic diversity observed in 180 accessions of *Aegilops* and *Triticum*, which originated from different eco-geographical areas of Iran, revealed that these germplasms are rich in unexploited potentially useful agronomic as well as morphological characters. Therefore, in regard with our finding showing remarkable levels of genetic diversity, it can be suggested that discovering this highly diverse gene pool may result in exploring valuable alleles for researches in wheat evolution, improvement, and adaptation.

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## ارزیابی تنوع آگرو-مورفولوژیکی در خویشاوندان وحشی گندم جمع آوری شده از ایران

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### چکیده

در این مطالعه یک مجموعه از ۱۸۰ اکسشن آژیلوپس و تریبتیکوم شامل شش گونه دیپلوئید ( *T. boeoticum* Bioss., *T. urartu* Gandilyan., *Ae. speltoides* Tausch., *Ae. Tauschii* Coss., *Ae. T. durum*, *Ae. neglecta* Req. ex ) پنج گونه تتراپلوئید ( *Ae. umbellulata* Zhuk. و *caudata* L. (*T. aestivum* L.) و یک گونه هگزا پلوئید (*Ae. crassa* Boiss و Bertol., *Ae. cylindrica* Host. جمع آوری شده از مناطق مختلف ایران بر اساس ۲۰ صفت زراعی و مورفولوژیک ارزیابی شده‌اند. نتایج آماری تفاوت معنی‌داری را بین اکسشن‌های مورد مطالعه نشان داد. پرامترهای ژنتیکی شانون-ویور  $(H'_{SW})$  و نی  $(H'_N)$  سطح متوسط تا بالایی از تنوع ژنتیکی درون گونه‌های آژیلوپس و تریبتیکوم نشان دادند. در تجزیه عاملی پنج مؤلفه نخست ۸۲/۱۷ درصد از کل واریانس ژنتیکی صفات آگرو-مورفولوژیکی را توجیه نمودند. با استفاده از تجزیه خوشه‌ای، بر اساس ۲۰ صفت اندازه‌گیری شده، ۱۸۰ اکسشن در دو گروه اصلی قرار گرفتند. علاوه بر این مطابق شکل دوبعدی ترسیم شده با استفاده از دو



تابع تشخیص اصلی گونه‌های مختلف درون شش گروه مجزا گروه‌بندی شدند به طوری که توزیع گونه‌ها درون هر گروه مطابق با ساختار ژنومی آن‌ها بود. به کلی نتایج این مطالعه سطح بالایی از تنوع ژنتیکی بین گونه‌های آژیلوپس و تریتیکوم بومی ایران را نشان داد که این تنوع می‌تواند برای برنامه‌های اصلاحی مدنظر قرار گیرد. از اینرو این نتایج ممکن است چشم‌انداز تازه‌ای در رابطه با ارتباط بین خویشاوندان وحشی و همچنین جستجو جهت یافتن صفات آگرونومی مطلوب برای بهبود و سازگاری گندم ارائه دهد.