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Evaluation of variability and genetic parameters in morphological traits *Agropyron trichophorum* using multivariate statistical analysis

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Abstract

The present investigation was carried out to estimate the genetic variability of morphological characters in 24 Accessions *Agropyron trichophorum* under irrigated condition. The results of variance analysis for seven measured traits showed significant differences among the examined characters indicating the presence of variability which can be exploited through selection. Comparison of means revealed that the genotypes 10(Semirom) and 23(Mazandaran) had the highest dry weight respectively. High heritability estimates were observed for number of shoot on bush and dry weight with indicating high genetic potential, low effect of environment and predominant role of additive gene effect on their expression. Correlation analysis demonstrated that dry weight is highly correlated with plant height, inter node length, flag leaf length and number of shoot on bush. Using principal component (PC) analysis, the first two PCs with eigenvalues more than 1 contributed 70.87% of the variability among accessions. pc1, which is the most important component, explained 44.02% of the total variation and was positively related to plant height, inter node length, number of spikelet on spike, flag leaf length and spike length; PC2 accounted for 26.85% of the total variation and the characters with the greatest weight on this component were Number of Shoot on Bush and Dry Weight. Forty *Agropyron* accessions were grouped into four clusters.

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Introduction

The distance between populations could be defined either on the basis of their pedigree relationship or their genetic composition. Diversity of origin or geographical diversity has been used as a measure of distance, in the absence of information regarding other criteria, to evaluate distance-heterosis correlations (Farshadfar, 2012). The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses and genetic erosion through time (Astley, 1992; Martiniello *et al.,* 1994). Distance analysis, a multivariate statistical technique based on the measurement of several metric characters, is an efficient method for evaluating genetic divergence or distance. Several reports on the application of distance analysis to the selection of parents in different crops have been published (Farshadfar and Farshadfar, 2004; Rao, 2003; Rao, 2004). Plant genetic resources for food and agriculture are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops, that are resistant to biotic and a biotic stresses and adapted to changing environments (Rao, 2004). *Agropyron*, as a range plant grows at the most of the rangeland of Iran, which have wide adaptation and grow in different climates. Therefore, gene pool conservation and its accurate application can be used in plant breeding programs in order to improvement rangeland and increasing forage production (Arghavani *et al.,* 2010). Gene pool of *Agropyron* includes about 19 species in Iran and 150 species in world (Bor, 1970). *Agropyron* has been applied in wide hybridization specially to transfer alien genes into cultivated wheat (Xu and Conner, 1994). Variablity among different wheatgrass (*Agropyron*) based on morphological and chemical traits were determined (Farshadfar and Farshadfar, 2004).

The objectives of the present experiment were to estimate (i) the genetic parameters for the study of genetic diversity in some morphological traits, and (ii) to study the association between the traits investigated and Dry Weight under irrigated conditions.

Materials and methods

Plant materials

In order to evaluation of genetic variation, 24 accessions of *A.trichophorum* were prepared from gene bank of Research Institute of Forests and Rangelands, Tehran, Iran (Table1).

The experiment was conducted using a completely randomized block design (RCBD) with three replications at Research field of Agricultural and Natural Resources Research Center of Kermanshah, Iran, during 2010-2011 growing season. Seeds were hand drilled and each genotype was sown in four rows of 4 m, with row to row distance of 0.50 m. The trials were hand-weeded twice and were grown under irrigated conditions. The Dry Weight (kg/h) was measured by harvesting each plot at crop maturity. Ten plants were randomly chosen from each plot to measure the Plant Height (PLH), Inter Node Length (INL), number of Spikelet on Spike (NSS), Flag Leaf Length (FLL), Spike Length (SPL) and Number of Shoot on Bush (NSB).

Statistical analysis

Variance analysis for Dry Weight and other related characteristics were performed over trails after verifying the homogeneity of trail variance error using Bartlett's test. Dunkan's multiple rang test values were calculated at the 5% probability level. The SAS (SAS Institute, Inc., 1997) procedures and programs were used for these calculations. After computing the correlation coefficients between all the characteristics, principal component (PC) analyses were performed using a combination of the methods described by Garcia Del Moral *et al.,* (1991) and Dofing and Knight (1992). Principal component analysis (Cattel, 1965) was performed using SPSS statistical program. Euclidean distance was determined among the genotypes and used for grouping of the genotypes by ward cluster method. MINITAB was used for clustering.

Estimation of biometrical genetic parameter

Various genetic parameters were estimated as follows (Farshadfar, 2013):

$$Ve = MSe$$

$$Vg = \frac{MSg - MSe}{r}$$

$$Vp = Vg + Ve$$

$$PCV = 100\sqrt{\sigma^2 p/\bar{X}}$$

$$GCV = 100\sqrt{\sigma^2 g/\bar{X}}$$

$$ECV = 100\sqrt{\sigma^2 e/\bar{X}}$$

$$h^2_{bs} = \sigma^2 g/\sigma^2 p$$

Where, V_e = environmental variation, MSe = error mean square, Vg = genotypic variation, r = number of replication, V_P = phenotypic after variation $\bar{X}$ is the mean, σ^2_g = is genetic variance, σ^2_p= is phenotypic variance, PCV= phenotypic coefficient of variation, GCV = genotypic coefficient of variation, ECV = environmental coefficient of variation and h^2_{bs} = broadsense heritability.

Results

Analysis of variance and mean comparison

The results of variance analysis (Table 2) for seven measured traits showed significant differences among the examined characters indicating the presence of variability which can be exploited through selection. The variability between genotypes was high for Dry Weight, Number of Shoot on Bush and Inter Node Length ($P<0.01$), while Plant Height, Number of Spikelet on Spike, Flag Leaf Length and Spike Length did not significantly differ between accessions ($P<0.05$) (Table 2). This indicated that differences existed between the accessions for many traits. Several researchers reported phenotypic divergence and extensive variation for morphologic traits in *Agropyron* germplasem (Farshadfar, 2012; Ray *et al.*, 1997; Vogel & Moore, 1998). Range and values of traits for *Agropyron* accessions are shown in Table 3. Dunkan's multiple rang test revealed that the accessions 10(Semirom) and 23(Mazandaran) had the highest Dry Weight respectively, while accessions 7(Bijar) and 19(Eghlid) exhibited lower value for these traits under irrigated condition. With regard to Table 3, it appears that accessions with higher Number of Shoot on Bush produce high Dry Weight.

Table 1. Gen bank cod and Origin of accessions of *A.trichophorum*.

Number	Origin	Gen bank cod	Number	Origin	Gen bank cod
1	Shahrekord	10-6	13	Salmas	2 Ghochi
2	Yasouj	13-13	14	Semirom	1-13
3	Esfahan	4007	15	Semirom	2-13
4	Semirom	3-13	16	Semirom	8-13
5	Chaharmahal	10-7	17	Yasouj	8-4
6	Karaj	6-13	18	Yasouj	6-8
7	Bijar	1 Bijar	19	Eghlid	8-7
8	Brojen	10-8	20	Eghlid	6-7
9	Eghlid	7-5	21	Tehran	316
10	Semirom	12-13	22	Esfahan	168
11	Esfahan	14-13	23	Mazandaran	313
12	Khoram-abad	11-4	24	Gorgan	314

Genetic variability

Table 4 represents various genetic parameters including phenotypic and genotypic coefficients of variation, broad sense heritability. Generally, the PCV and GCV were low for all the traits except Number of Shoot on Bush and Dry Weight with very high contribution of genetic portion. The variation observed between genotypes for the characters studied revealed that selection may be effective for the improvement of Dry Weight under irrigated conditions using morphological traits as a correlated response, although selection efficiency is related to the magnitude of heritability. High broad-sense heritability (Table 4) estimate was also observed for the characters Dry Weight (72%), and Number of Shoot on Bush (69%)., implying that the role of genetic variance was higher than environmental variance. High broad sense heritability indicates high genetic potentials for these traits, low effect of the environment and existence of predominant role of additive genes. High broad sense heritability seems to be a suitable basis for a reliable selection (Farshadfar *et al.,* 2013). Predictability of high performance and hence selection of materials based on the above criteria may lead to successful breeding program.

Table 2. Mean squares for 7 agronomic traits in 24 accessions *Agropyron*.

		Ms						
S.o.V	Df	PLH	INL	NSS	FLL	SPL	NSB	DW
Replication	2	38.48^{ns}	1.28^{ns}	0.48^{ns}	1.39^{ns}	3.72^{ns}	32.03*	93750*
Accessions	23	72.20*	18.55**	3.96*	3.49*	9.05*	53.74**	1521738**
Error	46	35.84	6.19	1.79	1.82	4.68	6.84	98343
(CV %)		7.67%	16.33%	12.24%	12.04%	14.54%	14.32%	10.65%

Correlation coefficient analysis

The correlation coefficients between Dry Weight and measured morphological traits are presented in Table 5. The Dry Weight had highly significant positive correlation with Plant Height, Inter Node Length, Flag Leaf Length and Number of Shoot on Bush. Among the traits, Number of Shoot on Bush had the strong association with seed yield, suggesting that these traits may be important yield predictors and perhaps it is the most important for yield improvement in *Agropyron*. Between the traits, strong positive correlation were observed between Number of Shoot on Bush and Dry Weight (0.544**).

Table 3. Mean of different morphological traits evaluated in 24 accessions *Agropyron*.

	Plh(cm)	Inl(cm)	Nss	Fll(cm)	Spl(cm)	Nsb	DW (kg.h)
1	69.01 e	16.70 abcdef	11.00 abcdef	11.11 abcde	15.21 abcde	20.47 b	2636 fgh
2	69.01 e	11.43 g	9.97 bcdef	10.38 bcde	13.01 abcde	17.53 bcde	2693 efg
3	73.24 cde	17.02 abcd	11.20 abcdef	11.99 abcd	16.89 ab	18.27 bcd	2995 def
4	79.03 bcde	15.39 abcdefg	11.27 abcdef	11.40 abcde	15.75 abcde	17.13 bcde	2762 def
5	80.61 abcde	14.82 abcdefg	11.27 abcdef	11.39 abcde	15.27 abcde	20.27 b	3590 bc
6	77.91 bcde	12.99 bcdefg	11.37 abcde	10.22 bcde	12.22 de	15.53 bcde	2097 hi
7	74.07 cde	14.63 abcdefg	11.10 abcdef	11.35 abcde	17.14 ab	14.80 cde	1530 j
8	78.05 bcde	17.61 ab	12.60 ab	12.25 abc	17.27 a	15.67 bcde	2794 def
9	72.41 cde	12.43 cdefg	10.60 abcdef	9.81 cde	12.29 cde	16.67 bcde	2977 def
10	86.71 ab	18.89 a	11.47 abcd	12.83 ab	15.67 abcde	19.67 bcde	4556 a
11	77.92 bcde	18.61 a	12.67 a	11.49 abcde	16.95 ab	17.33 bcde	3708 b
12	78.89 bcde	12.40 defg	10.27 abcdef	10.53 abcde	13.52 abcde	17.33 bcde	2925 def
13	74.03 cde	14.18 abcdefg	8.80 ef	9.45 de	15.47 abcde	19.00 bcd	3356 bcd
14	77.85 bcde	16.97 abcd	10.40 abcdef	12.86 ab	16.33 abcd	18.67 bcd	3294 bcde
15	84.01 abc	17.62 ab	11.20 abcdef	11.71 abcd	13.90 abcde	14.67 de	3365 bcd
16	78.36 bcde	12.87 bcdefg	8.66 f	10.77 abcde	12.87 bcde	17.80 bcde	2545 fghi
17	78.78 bcde	12.10 efg	9.86 cdef	10.71 abcde	13.29abcde	15.80 bcde	3049 cdef
18	80.31 abcde	17.17 abcd	9.46 def	10.49 abcde	13.81 abcde	20.53 b	2160 ghi
19	78.27 bcde	11.92 fg	10.51 abcdef	11.39 abcde	14.53 abcde	17.73 bcde	2001 i
20	79.30 bcde	18.66 a	11.67 abcd	11.27 abcde	16.94 ab	16.07 bcde	2772 def
21	78.00 bcde	15.59 abcdefg	12.27 abc	11.48 abcde	14.98 abcde	19.93 bc	2753 def
22	70.99 de	11.78 g	9.73 cdef	8.82 e	11.67 e	12.97 e	2602 fgh
23	82.06 abcd	17.30 abc	12.20 abc	12.47 abc	15.76 abcde	25.80 a	4525 a
24	90.71 a	16.83 abcde	12.80 a	13.15 a	16.57 abc	18.87 bcd	3054 cdef

Principal component analysis

Principal component analysis can determine which of the characters most strongly contributes to the PC. The analyses reduced the original 7 characters in the experiment to 2 PCs. The first 2 PCs with eigenvalues >1 explained 70.87% of the variation among the 24 accessions (Table 7). Other PCs had eigenvalues <1 and have not been interpreted. PC1, which is the most important component, explained 40.02% of the total variation and was positively related to plant height, , inter node length, number of spikelet on spike, flag leaf length and spike length; PC2 accounted for 26.85% of the total variation and the characters with the greatest weight on this component were Number of Shoot on Bush and Dry Weight (Fig 1).

Table 4. Estimate of genetic parameters for the studied traits.

h^2_{bs}	PCV	GCV	Ve	Vg	Vp	Traits
25.27	8.87	4.46	35.84	12.12	47.96	Plh(cm)
39.93	21.07	13.31	6.20	4.12	10.32	Inl(cm)
28.82	14.51	7.79	1.79	0.72	2.51	Nss
23.36	13.75	6.65	1.83	0.56	2.38	Fll(cm)
23.69	16.65	8.10	4.69	1.46	6.14	Spl(cm)
69.54	21.64	25.95	6.85	15.63	22.48	Nsb
72.44	25.72	23.41	98343	474465	572807	DW (kg.h)

Table 5. Simple coefficient correlation between agronomic traits in 24 accessions Agropyron.

	Plh(cm)	Inl(cm)	Nss	Fll(cm)	Spl(cm)	Nsb
Inl (cm)	0.436*					
Nss (cm)	0.410*	0.604**				
Fll	0.640**	0.691**	0.657**			
Spl (cm)	0.248	0.740**	0.594**	0.707**		
Nsb (cm)	0.239	0.293	0.211	0.362	0.212	
DW (kg.h)	0.423*	0.456*	0.289	0.407*	0.220	0.544**

Cluster analysis

WARD hierarchical clustering for grouping accessions based on Euclidean distance coefficient (Fig. 2) were identified the four distinctive groups.

First cluster

Accessions 1(Shahrekord), 2(Yasouj), 3(Esfahan), 4(Semirom), 8(Brojen), 9(Eghlid), 12(Khoram-abad), 16(Semirom), 17(Yasouj), 20(Eghlid), 21(Tehran), 22(Esfahan) and 24(Gorgan) were classified in the first cluster including 54.16% of total accessions. Which the average Dry Weight was 2811.78 (kg/h) for this group.

Table 6. Principal components (PCs) for 7 morphological traits in 24 accessions *Agropyron.*

Extraction	PC2	PC1	Traits
0.46	0.47	0.48	Plh(cm)
0.76	0.29	0.82	Inl(cm)
0.68	0.13	0.81	Nss
0.82	0.36	0.83	Fll(cm)
0.77	0.03	0.88	Spl(cm)
0.70	0.83	0.08	Nsb
0.76	0.85	0.21	DW (kg.h)
	1.88	3.08	Eigen Value
	26.85	44.02	% variance explained
	70.87	44.02	% of cumulative

Second cluster

Accessions 6(Karaj), 7(Bijar), 18(Yasouj), and 19(Eghlid) were classified in this cluster including 16.6% of total accessions. Which the average Dry Weight was 1946.70 (kg/h) for this group, values of Dry Weight in this cluster were less than the total means (Table 5).

Table 7. Comparing the characteristics of the groups Accessions in cluster analysis.

Group	Plh(cm)	Inl(cm)	Nss	Fll(cm)	Spl(cm)	Nsb	DW (kg.h)
C_1	76.85	14.67	10.60	11.05	14.42	17.26	2811.78
C_2	78.88	14.17	10.91	10.86	15.58	17.15	1946.70
C_3	77.63	16.44	10.86	11.40	14.63	17.98	3462.16
C_4	84.38	18.09	11.83	12.67	15.71	27.73	4540.58

Third cluster

Accessions 5(Chaharmahal), 11(Esfahan), 13(Salmas), 14(Semirom) and 15(Semirom) were classified in this cluster accounting for 20.83% of the total accessions. Which the average Dry Weight was 3462.16 (kg/h) for this group.

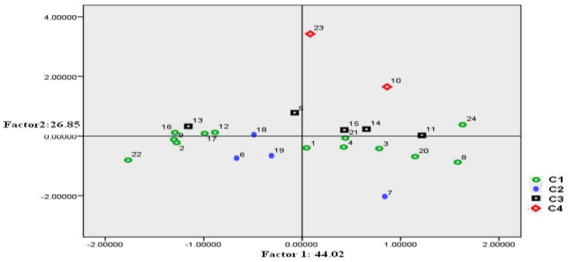

Fig. 1. Scatter plot for accessions based on two first axes from Principal component analysis.

Fourth cluster

Accessions 10(Semirom) and 23(Mazandaran) were classified in this cluster accounting for 8.33% of the total accessions. Which the average Dry Weight was 4540.58 (kg/h) for this group, values of Dry Weight in this cluster were greater than the total means (Table 5).

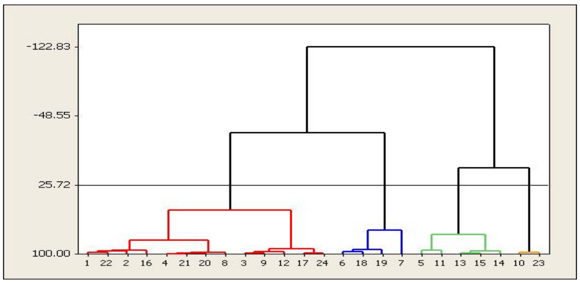

Fig. 2. Dendrogram of the *Agropyron* accessions based on the dissimilarity matrix developed using morphological markers.

Therefore considering of genetic distance between these groups, using of the third and fourth group accessions can be useful in breeding programs, to utilization of heterosis.

Discussion

The description of agronomically important and useful characteristics is an important prerequisite for effective and efficient utilization of germplasm collections in breeding programs (Duvick, 1984; Naghavi and Jahansouz, 2005). An *Agropyron* accessions collection has been assembled and we have shown that there is a high level of morphological diversity for most of the traits observed, which may be useful for future breeding endeavors. The results of variance analysis showed significant differences in terms of all traits in the irrigated conditions. Accessions 10(Semirom) and 23(Mazandaran) had the highest Dry Weight and Number of Shoot on Bush while accessions 7(Bijar) and 19(Eghlid) exhibited lower value for these traits under irrigated conditions. This indicates that the magnitude of differences in cultivars was sufficient to select those irrigated conditions. In general, this genotypic variation in these characteristics may be attributed to differences in the ability of the variation to absorb more water from the soil and or the ability to control water loss through the stomata's. The results of present study showed that even through the relationships (correlations) among some characters were significant. According to these results, linear relations among examined characters are insufficient in plant breeding programs. Variability study indicated that

the materials in the present investigation possessed moderate variability for the characters Number of Shoot on Bush and Dry Weight. The traits Dry Weight and Number of Shoot on Bush exhibited high heritability. Therefore, it is concluded that these traits had to be accounted for direct selection for the improvement of Dry Weight. Multivariate methods of analysis, such as the PC and cluster analyses used in the present study, have shown the germplasm groupings in many genetic resources such as *Ag. Cristatum* and *Ag. Desertorum* (Taghizadeh, 2008).
In our analysis, we were able to define groups of accessions that were significantly different from each other for characters of interest. We found that there was no relationship between genetic divergence and geographical origins because accessions from one place entered into more than one cluster. Conversely, accessions from different geographical origins were relatively unique and tend to be clustered in one part of the dendrogram, which information suggests that the morphological variation analyzed is determined not only by environmental differences but also by genetic factors. This study has implications not just for the origin of this crop, but also for the management of genetic resources and their uses in applied breeding programmers, particularly for the development of a core collection. Information about current genetic diversity permits the classification of our available germplasm into various/heterotic groups, which is particularly important to hybrid/cross-breeding programs in *Agropyron*.

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