DETERMINING DIVERSITY AND GENETIC DISTANCE OF 31 GENOTYPES OF *AGROPYRON DESERTORUM* FOR GRAIN YIELD AND ITS COMPONENTS USING MULTI-VARIATE STATISTICAL METHODS

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ABSTRACT

In order to investigate diversity and explain the relations of grain yield with its components, 31 genotypes of *Agropyron desertorum* were densely planted in randomized complete blocks design with four replications in climatic conditions of Arak city (Markazi Province, Iran). Some of the most important morphological and physiological attributes were measured. The data collected in two years were analysed; principal components analysis and cluster analysis were used to determine genetic distance and genotype classification. In principal components analysis, the specific values obtained from 1 to 5 components were larger than one. Totally, 75 percents of all variances justified the variables. In the first component, attributes of ear emergence time, pollination time, plant height, and forage yield, and in the second component, attributes including number of stems, grain yield, and harvest index had greater specific vector coefficients besides major attributes for genotype classification. In cluster analysis, genotypes were categorized in seven groups. According to the obtained results, clusters 4, 6 and 7 - in term of grain yield - and clusters 5, 6 and 7 - in terms of forage production - were significantly different from other groups. Genotypes 3477P4 and 341P11 belonging to cluster 7 were known as superior genotypes in terms of forage production and two genotypes 341M and 287P8 belonging to cluster 4 were proved to be the best genotypes in terms of grain production. Good agreement was observed between the results obtained from cluster and principal components analyses in scatter diagram representation of 31 genotypes based on two principal components (first and second) using fourteen studied attributes.

Key words: *Agropyron desertorum*, grain yield, yield components, principal components analysis, cluster analysis.

INTRODUCTION

*A*gropyron desertorum is one of the valuable fodder Graminaes for creating pastures and for forage production. Plants of *Agropyron* type have good compatibility with dry Mediterranean climatic conditions like that of Iran (Cerpo, 2000). In order to maximally benefit from dry matter production, the plant is normally cropped after flowering and just before start of pollination. Harvesting later than this stage, despite increase in forage yield, would result in sever worsening of forage grass quality (Jafari et al., 2003).

The plants of this species are outcrossing and tetraploid having 28 chromosomes plus one additional chromosome (Asghari et al., 2007). These plants have originated from central Asian plains; they feature other desirable characteristics such as durable drought persistence (Mellish et al., 2002). Today, along with forage yield enhancement, grain production of forage plants is also particularly significant. Prior to implementation of selective modification plans, it is required to know the relationship between grain yield and its components; because success in modification and production of highly-reproductive cultivars depends upon identification of genetic control of grain yield and its relationship with other yield components and morphological attributes (Jafari & Rezaeifard, 2010). Although correlation coefficients are widely used for determining and explaining the degree of relationship between two attributes, they might become deceptive since good correlation between two attributes may have

resulted from indirect effects of other attributes, and using simple correlation analysis might not generally explain the relationships between attributes (Ali et al., 2003). Therefore, it is necessary to differentiate the direct and indirect effects of attributes affecting the yield. Path analysis enables us to distinguish the direct impact of each yield component from indirect effects caused by mutual relationships among them (Emam & Borjan, 2000). For the same purpose, plant modification experts utilize path analysis method as a technique for determining significance of attributes affecting the yield (Jafari et al., 2003).

Selection with generation testing is one of the breeding methods of forage plants. Success in selection is dependent on the diversity that creates new genetic combination and heterosis. Heterosis likelihood in anastomosis plants goes up by increasing the genetic distance between genotypes (Humphreys, 1991). Genotypes classification based on genetic distance is effective in a breeding plan only when several attributes are simultaneously investigated. Therefore, following the identification of correlation level of respective attributes and differentiation of direct and indirect effects of most important influential attributes on grain and forage yields, using standardized variables, cluster analysis with Ward method and Euclidian distance scale was conducted with the intention of determining the genetic diversity pattern, genotypes classification and specifying the genetic distance between them.

MATERIAL AND METHODS

31 genotypes of *Agropyron desertorum* were collected from natural resource genetic bank of Iran's Forests and Forages Research Organization. The experiment plot was located in the research field of Islamic Azad University of Arak City (with longitude: 50°57`, latitude: 34°33' and altitude 1760 m from free sea level). It was disked in autumn 2003 following composting with 200 kg phosphate and 100 kg nitrogen fertilizers per hectare. The test was implemented at the density of 10 kg of grain per hectare and in randomised complete blocks design with four replications. The experimental plots had 1*2 meters dimensions consisting of four 2-meter lines, 25 cm apart from each other. In this research, attribute measurements were conducted for two years as follows:

1) Ear emergence time: based on number of days from 21 March until emergence of one third of ears in each plot

2) Pollination time: based on number of days from 21 March until emergence of anthers in one third of each plot

3) Plant height: 10 plants were randomly selected in each plot and the longest stem was chosen in each plant; the height was measured in centimetres and the average was recorded as the plant height

4) Number of stems: 10 plants were randomly selected in each plot and the average number of reproductive tillers was identified.

5) Forage yield: after cutting forage of each plot from height of 6cm, the samples were dried in oven at 75° C for 12 hours and then weighed and expressed in tones per hectare.

6) Ear length: 10 plants were randomly selected from each plot and the average length of ears was measured with a ruler in centimetres.

7) Number of grains in ear: 10 plants were randomly selected from each plot and number of grains was counted and averaged.

8) Grain weight in ear: after cleaning and winnowing ten ears in each plot, the average grain weight was computed in each ear.

9) Number of spikelets in ear: 10 plants were randomly selected from each plot and number of spikelets was counted and averaged.

10) Weight of 100 grains: was evaluated by counting and weighing 1000 grains by grain-counter machine

11) Flag leaf surface area: 10 plants were randomly selected from each plot and average of multiplication of width by length of the flag leaf was computed in square centimetres.

12) Peduncle length: Average distance between ear and flag leaf and in each plant was measured in centimetres for 10 selected plants in each plot.

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13) Grain yield: after cutting and drying all plants of each plot, and threshing and separating the chaffs and straws, the grain weights were measured in kg hectare^{-1}.

14) Harvest index: was obtained by dividing grain weight of each plot to the weight of aerial biomass.

Statistical Analyses

Simple phenotypic correlation coefficients between fourteen attributes and stepwise regression coefficients for identifying significant attributes affecting grain yield were computed (Table 1). Path analysis was subsequently performed to identify the direct and indirect effects of significant attributes imposed to regression model (Table 2). Principal components analysis (PCA) using 14 attributes was applied for 31 genotypes for determining the contribution of each attribute to total variation, in order to reduce data volume, and allow better interpretation of relations. The scatter diagram of genotypes was plotted on two principal components (Table 3 and Figure 2). Finally, cluster analysis was conducted using standardized variables with Ward method and Euclidian distance scale, in order to determine the genetic diversity pattern, classify the genotypes, and specify the genetic distance between them (Tables 4 and 5 and Figure 1). The software SAS.9.2, JMP and Path.2 were used to statistically analyse the data.

RESULTS AND DISCUSSION

Correlation between attributes

Explaining the relationship between grain yield and morphological attributes is highly important in implementation of selection plans. For this reason, correlation coefficients between grain yield and other attributes were computed. The correlation between grain yield and number of stems per plant was positive and significant at 1% probability level. In other words, a higher capability to produce reproductive stems would bring about higher grain yield in a genotype. Results of binary phenotypic correlation analysis between averages of attributes are shown in Table 1.

Table 1. Correlation analysis between 14 traits in 31 genotypes of *Agropyron desertorum*

Traits	Spike Appearance date	Pollination date	Plant height	Tiller number	DM yield	Spike length	Leaf size	Seed weight per spike	Seed yield	Seed no. per spike	Spikelet no. per spike	Thousand grain weight	Harvest index
Pollination date (day)	**0.91****												
Plant height (cm)	**-0.51****	**-0.44****											
Tiller number (No/m^2)	-0.03	-0.10	0.17										
DM yield (t/ha)	-0.25	**-0.40***	**0.60****	**0.46****									
Spike length (cm)	-0.12	-0.10	-0.03	0.03	0.21								
Leaf size (cm^2)	0.18	0.28	-0.01	0.21	-0.20	-0.09							
Seed weight per spike (g)	-0.24	-0.29	0.26	-0.19	**0.36***	0.18	**-0.45****						
Seed yield (kg/ha)	0.00	-0.05	0.02	**0.79****	**0.44****	0.28	0.01	0.16					
Seed number per spike	-0.12	-0.23	0.17	-0.09	**0.34***	0.26	**-0.39***	**0.71****	0.07				
Spikelet number per spike	-0.16	-0.22	-0.10	0.08	-0.04	0.26	-0.14	0.14	**0.31***	0.05			
Thousand grain weight	-0.08	-0.01	0.15	-0.04	0.06	-0.04	-0.12	0.29	0.18	**-0.34***	0.06		
Harvest index (%)	0.28	0.13	-0.23	**0.52****	**-0.36***	-0.06	0.01	-0.12	**0.66****	-0.12	0.26	0.05	
Length of peduncle (cm)	-0.04	0.07	0.08	-0.06	0.05	0.20	0.17	0.13	-0.06	-0.14	-0.14	0.25	-0.29

*significant at the 0.05 probability level, ** significant at the 0.01 probability level.

Similar positive correlation between grain yield and number of stems per plant was reported in *Setaria sphacelata* by Hacker and Cauny (1997), in *Dactylis glomerata* by Jafari and Naseri (2007), and in *Bromus catharitcus* by Abbott et al. (2007). Also, correlation coefficients between grain yield and other attributes including harvest index, number of spikelets in ear, number of stems, and forage yield were positive and significant. Such correlation is expected taking into account that harvest index is obtained from the ratio of grain yield to biomass yield. Also, the positive and significant relationship between forage yield and plant height, number of reproductive stems, number and weight of grains in ear, and grain yield indicate the significance of these attributes in forage yield improvement. Negative correlation of forage yield with pollination time is indicative of the fact that the sooner a genotype starts its pollination, the higher is forage yield. Similar results were reported by Jafari et al. (2006) in tall fescue and by Jafari & Naseri (2007) in *Dactylis glomerata*.

Regression analysis

In stepwise regression analysis, grain yield was treated as the dependent variable of function (Y) and the attributes: number of stems (X_1), harvest index (X_2), forage yield (X_3) and ear length (X_4) were regarded as independent variables; the following equation was obtained:

$$Y = -383.25 + 0.38X_1 + 19.10X_2 + 93.9X_3 + 21X_4$$

The significant determination coefficient ($R^2 = 0.90$) observed in the above regression equation indicates effectiveness of these attributes in grain yield improvement. As observed, all attributes X_1-X_4 affected grain production with positive coefficient. Comparison of regression and correlation coefficients is suggestive of agreement between regression and correlation analyses reconfirming the significance of aforementioned attributes in enhancement of grain and forage yields in *Agropyron desertorum*. Similar to these results, Jafari and Naseri (2007) reported a positive and significant correlation between grain and forage yields of *Dactylis glomerata* in which grain yield was function variable and other attributes were supposed as independent variables.

Path analysis

In path (causality) analysis, grain yield was similarly supposed as the function variable and the rest four attributes in the stepwise regression model (number of stems, harvest index, forage yield and ear length) were introduced as independent variables (Table 2). As the path analysis results suggest, harvest index showed the greatest direct effect on grain yield; its total effect was 0.66. After harvest index, forage yield and number of reproductive stems assumed the largest direct effects on grain yield improvement. Number of reproductive stems exhibited the largest indirect effect (through improvement of harvest index) on grain yield. Furthermore, forage yield, in additions to its direct effect, indirectly influenced grain yield enhancement (through increasing number of stems).

Table 2. Path coefficients of direct and indirect effects of the traits that were included in stepwise regression model with seed yield

Traits	Direct effect	Indirect effect				Total effect correlation
		Tiller number	Harvest index	DM yield	Spike length	
Tiller number	0.310		**0.332**	0.184	-0.030	0.79
Harvest index	**0.63**1	0.163		-0.132	-0.002	0.66
DM yield	**0.518**	**0.202**	-0.062		-0.041	0.42
Spike length	-0.06	0.191	0.015	0.112		0.26

Residual Error = 0.35

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Principal components analysis

The parameters obtained from principal components analysis including specific values, percentage of justified variance, and specific vector coefficients for components 1 to 4 can be seen in Table 3. In principal components analysis, the specific values of components 1 to 5 were larger than 1 and respectively accounted for 25, 18, 13, 10 and 9 percents of all variances of variables (in total 75%). Relative values of specific vector coefficients of the first component showed that ear emergence time, pollination time, plant height and forage yield were the most important attributes for genotypes classification. In the second component, number of stems, grain yield and harvest index had larger specific vector coefficients. According to the results, the first component was designated phenology component and forage yield, and the second component was referred to as grain yield component. Diverse coefficients of specific vectors in independent components indicated that it is possible to improve forage and grain yields in genotypes of *Agropyron desertorum,* via selecting different combinations of these attributes.

Table 3. Eigen value, Percent of Total Variance and Cumulative Variance Percent related to specific traits of each of the principal components analysis

Traits	Prin 1	Prin 2	Prin 3	Prin 4	PCA 5
Date of spike appearance	**-0.42**	0.08	-0.23	0.16	0.34
Date of pollination	**-0.41**	0.03	-0.09	0.23	0.37
Plant height	**0.34**	-0.06	**0.33**	-0.06	0.11
Number of fertile stems	0.08	**0.51**	0.26	-0.15	0.18
DM yield	**0.40**	0.00	0.22	-0.08	0.28
Spike length	0.25	0.25	-0.06	0.17	0.10
Size leaf	-0.24	0.08	**0.44**	-0.10	0.13
Seed weight per spike	0.34	-0.09	**-0.35**	**0.34**	0.15
Seed yield	0.13	**0.57**	0.00	0.12	0.16
Seed number per spike	0.29	-0.07	**-0.45**	-0.14	**0.43**
Spikelet number per spike	0.11	0.21	-0.25	0.02	**-0.49**
Thousand grain weight	0.08	0.04	0.14	**0.65**	-0.28
Harvest index	-0.12	**0.52**	-0.18	-0.01	-0.13
Length of peduncle	0.03	-0.11	0.29	**0.52**	0.18
Eigen Value	3.50	2.58	1.78	1.46	1.27
Percent of Total Variance	25.00	18.46	12.75	10.46	9.10
Cumulative Variance Percent	25.00	43.46	56.20	66.66	75.76

The underlined and bold data have higher values in the PCA axes

Cluster analysis

Cluster analysis with Ward method was carried out to determine the genetic diversity pattern, classify the genotypes and specify the genetic distance between them. All fourteen attributes of 31 genotypes were utilized in cluster analysis. Genotypes were categorized in 7 different clusters with dendrogram cutting at a Euclidian distance of 3.94. The clusters 1-7 incorporated 1, 5, 2, 6, 8, 4 and 5 genotypes respectively (Table 5 and Figures 1 and 2). Scatter diagram of 31 genotypes of *Agropyron desertorum* based on two principal components is illustrated in Figure 1. The first component played remarkable role in distinction of groups regarding ear emergence time, pollination time, plant height and forage yield. Specific vector coefficients of ear emergence and pollination times were negative while those of forage yield and plant height were positive. Consequently, genotypes belonging to cluster 7 are long-duration and the most prolific genotypes, while those belonging to clusters 1 and 3 are short-duration and the least prolific genotypes. In the second component, number of

reproductive stems, grain yield and harvest index having positive sign contributed to differentiation of groups in such a way that genotypes of cluster 4 had large number of reproductive stems, desirable grain yield and high harvest index. As the results indicate, genotypes of cluster 4 and then those of cluster 7 were known as superior genotypes in terms of grain production. Also, genotypes 3477P4 and 341P11 belonging to cluster 7 were proved to be the best in terms of forage production (Figure 2). There was a good correlation between cluster analysis and principal components analysis in genotypes scattering based on the two first components.
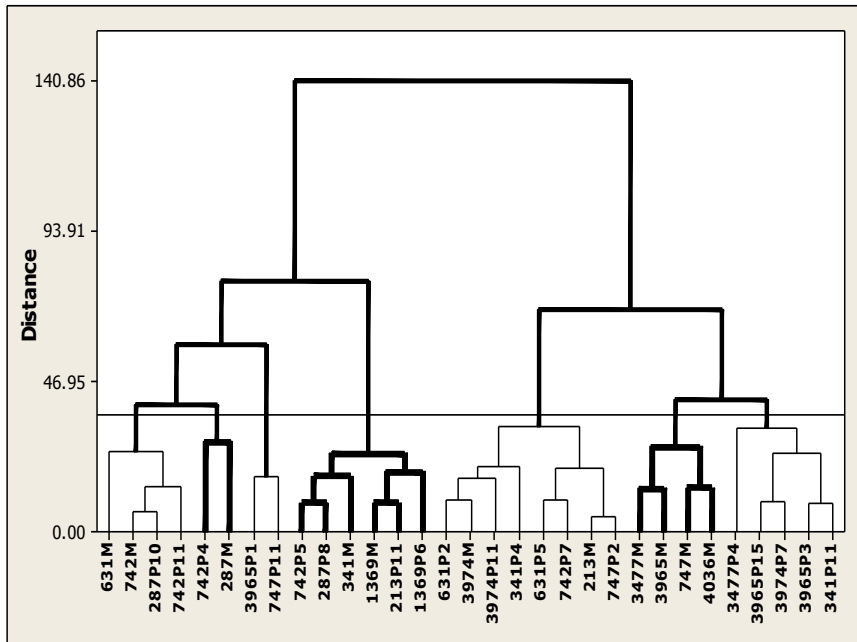

Figure 1. Dendrogram of 31 genotypes of *Agropyron desertorum* by cluster analysis using Ward analysis method on 14 traits
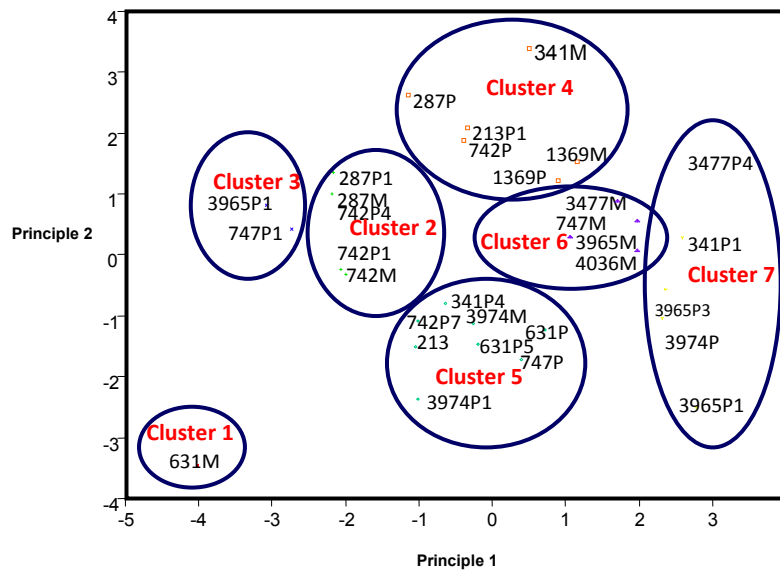

Figure 2. Scatter plot of 31 genotype *Agropyron desertorum* for the first two principal components

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Table 4. Names of *Agropyron desertorum* genotypes classified in each of the clusters

Cluster Number						
1	2	3	4	5	6	7
631M	742M	3965P1	742P5	631P2	3477M	3477P4
	742P11	747P11	1369M	631P5	3965M	3965P15
	742P4		1369P6	742P7	747M	3965P3
	287M		287P8	213M	4036M	341P11
	287P10		213P11	341P4		3974P7
			341M	747P2		
				3974M		
				3974P11		

Table 5. Means of traits used in the 7 clusters classification

Cluster	Spike emergence date	Pollination date	Plant height	Tiller number	DM yield	Spike length	Leaf size
1	47.67 [a]	75.58 [a]	39.38 [d]	87.20 [c]	1.62 [d]	5.50 [c]	3.44 [a]
2	46.92 [ab]	74.55 [ab]	41.58 [dc]	141.30 [ab]	1.94 [dc]	6.46 [ab]	2.78 [b]
3	47.46 [a]	75.59 [a]	45.46 [abc]	184.20 [a]	2.29 [bc]	6.02 [bc]	4.57 [a]
4	45.61 [bc]	73.50 [bc]	43.66 [bcd]	179.57 [a]	2.25 [bc]	6.85 [a]	2.97 [b]
5	45.26 [bc]	73.59 [bc]	45.73 [abc]	127.47 [bc]	2.40 [bc]	6.50 [ab]	2.63 [b]
6	42.92 [d]	70.96 [d]	47.60 [ab]	171.83 [a]	2.60 [ab]	6.46 [ab]	2.58 [b]
7	44.38 [dc]	72.90 [c]	49.04 [a]	154.00 [ab]	2.98 [a]	6.85 [a]	2.79 [b]
Mean	45.37	73.470	45.21	152.14	2.38	6.54	2.90
LSD	3.24	3.25	7.52	58.42	0.96	0.66	1.84
F	8.91 **	10.85 **	5.45 **	5.30 **	7.78 **	3.27 *	3.85 **

Continue Table 5

Cluster	Seed weight per spike	Seed yield	Seed no. per spike	Spikelet per spike	Thousand grain weight	Harvest index	Peduncle length
1	0.15 [b]	162.17 [c]	68.84 [b]	31.73 [a]	2.12 [a]	7.60 [b]	15.55 [a]
2	0.16 [ab]	348.66 [b]	74.01 [ab]	32.22 [a]	2.22 [a]	16.53 [a]	14.02 [a]
3	0.14 [b]	393.33 [ab]	68.22 [b]	28.29 [b]	2.12 [a]	14.54 [a]	16.98 [a]
4	0.18 [ab]	466.39 [a]	74.56 [ab]	33.73 [a]	2.46 [a]	16.29 [a]	15.74 [a]
5	0.17 [ab]	297.08 [b]	74.56 [ab]	31.29 [ab]	2.34 [a]	10.59 [b]	16.72 [a]
6	0.18 [ab]	387.92 [ab]	78.62 [ab]	31.06 [ab]	2.24 [a]	14.43 [a]	15.45 [a]
7	0.20 [a]	368.97 [ab]	94.77 [a]	32.74 [a]	2.24 [a]	10.58 [b]	15.89 [a]
Mean	0.17	363.34	31.94	31.937	2.297	13.30	15.77
LSD	0.03	142.41	17.28	3.74	0.44	5.13	4.44
F	2.60 *	7.75 **	3.37 *	3.37 *	0.94 ns	10.96 **	0.88 ns

* and ** significant at the 0.05 and 0.01 probability level, respectively.
Means followed by the same letters in each column are not significantly different ($P < 0.05$).

CONCLUSIONS

We recommend to use cultivars with high grain and forage yields for producing synthetic cultivars and to create heterosis via anastomosis between genotypes featuring desirable attributes, which possess the largest portion in principal components analysis and greatest genetic distance in cluster analysis.

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