Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2013, 4 (5):175-182
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Genetic variation and interrelationship of seed mucilage, swelling factor and agronomic traits in *Plantago ovata* wild accessions

D. Karimi[1], B. Heidari[1]*, S. N. Daneshnia[2] and A. Dadkhodaie[1]

[1]*Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Iran*
[2]*Department of Plant Protection, College of Agriculture, Shiraz University, Iran*

ABSTRACT

Wild accessions are raw reservoirs of genetic diversity that constitute a wide germplasm harboring unique genes and characters that can be used in developing new varieties. Seven wild P. ovata accessions, collected from different climates of south of Iran were selected for evaluation of mucilage content, swelling factor, morphological traits and antioxidants. Although the mean of square for mucilage content was not significant, it ranged from 0.10 g in the accessions collected from Darab to 0.15 g in Abadeh and Firuzabad. Seed swelling factor averaged 11 ml, was in the range of 8.2 to 16.7 ml. Grain yield, averaged 4.9 g, ranged from 3 to 7.8 g in Jahrom and Marvdasht, respectively. The highest plant height was observed in Marvdasht (35.3 cm), Fasa (35.0 cm) and Abadeh (34.5 cm) while Darab (29.2 cm) was the shortest. The highest genetic coefficients of variation were observed for spike number (50.7%), catalase enzyme (47%), spike length (43%) and leaf area (43%). Among mucilage parameters, seed swelling factor (24%) was highly variable. Correlation analysis indicated that seed swelling factor (r=-0.35) was negatively correlated with grain yield although mucilage content (r= 0.36) had positive correlation with grain yield. The wild accessions classified into three major groups based on their similarities in cluster analysis. Seed swelling, grain yield, antioxidants and morphological traits were highly heritable with heritability estimates above 70%. In conclusion, the wild P. ovata genotypes were highly variable for grain yield, morphological traits and seed swelling factor and identification of different wild accessions provides useful information for breeding P. ovata varieties for various purposes.

Keywords: Genetic variation, Grain yield, Mucilage, *Plantago*, Swelling factor

INTRODUCTION

Plantago ovata is important for its mucilage content of the seed that mainly used in industry and medicine [25]. Mucilage is a thick, gluey substance produced by some plants and microorganisms. It is a polar glycoprotein and an exopolysaccharide. Mucilage in plants plays a role in the storage of water and food, seed germination, and thickening membranes. Mucilage can be used in gastrointestinal inflammatory processes, associated with topical irritation agents [9, 16, 19]. Natural mucilage is preferred over semi-synthetic and synthetic materials due to their non-toxic, low cost, free availability, emollient and non-irritating nature. The mechanism of action is that mucilage covers the mucous membranes and prevents irritation of the nerve endings. Mucilage which is edible is used in medicine for its demulcent properties. Mixed with water, mucilage is used as glue, especially for bonding paper items such as labels, postage stamps, and envelope flaps. Differing types and varying strengths of mucilage can also be used for other adhesive applications, including gluing labels to metal cans, wood to china, and leather to pasteboard.

Plants of the family Plantaginacea, highly recognized medicinal plants, rich in mucilage [2, 18, 24]. The genus *Plantago*, belonged to the family Plantaginacea, has been recognized with over 200 different species. *Plantago* center of diversity is mostly in the temperate and tropical regions and central Asia [11, 16, 18]. The plants of this genus are small annual herbs with medicinal values and useful metabolites in the seeds. The seed husk of the species *Plantago ovata* which is the only cultivated species is called psyllium in English, Isabgol in Hindi and Isfarzeh in Persian [16]. The husk which is about 25 to 30% of the seed of *Plantago* has inflammatory activity and used in irritation of the intestinal tract [2, 16, 19].

For the purpose of genetic conservation and collecting raw germplasm for a successful breeding program, identification and clustering of the genotypes are of the most important. Characterizing the genotypes for their genetic variability is necessary for the purpose of breeding *Plantago* for quality mucilage, swelling factor and grain yield. There are few reports on *Plantago* genetic variations showing the urgent need for germplasm identification for defining varieties of the crop. Van Dijk [7, 8] emphasizes that leaf morphology, seed size and production are important for adapting *Plantago* species to the respective habitat conditions. Rohilla et al. [2] clustered *Plantago ovata* genotypes, commercially known as Blond psyllium [14], into three major groups comprising ten, seven and one genotypes. In the study reported by Das [15], the seed swelling factor of *Plantago indica* was not influenced by sowing date and spacing. On the other side, sowing *P. ovata* during the first week of December was found to be ideal for Jammu conditions (India) which was a different story than the report of Koul and Sareen [1] indicating mid-October to mid-November as the ideal sowing date. In another work, mucilage content varied from 0.05 to 0.10% in *P. ovata* accessions, although the mean of squares for genotypes were not significant for mucilage content and seed inflation factor [3]. There are evidences showing that the seed mucilage's of some of *Plantago* species exhibit good binding properties and affect retardation of drug dissolution [18]. Evaluation of British and European *Plantago* collections indicated that the species *major* and *intermedia* have variations for ozone (O_3) resistance [13].

Therefore, given the wide variation of *Plantago* genotypes for different applications in medicine and industry uses, the present study was conducted to evaluate *P. ovata* wild accessions (Table 1) to characterize genetic variations for mucilage quality, swelling factor and morphological traits and to estimate heritability of the traits. Identification of wild genetic reservoirs and heritability information helps breeders to draw an efficient program for using in breeding plans with the aim of developing new varieties.

MATERIALS AND METHODS

Seven wild *P. ovata* accessions, collected from different climates of southwest of Iran (Table 1), were selected for evaluation of mucilage content, swelling factor, morphological traits and antioxidants. The experiment was arranged as a randomized complete block design (RCBD) with three replications in the Agricultural Research Station of the College of Agriculture, Shiraz University, Iran. Prior to sowing, the seeds were washed under faucet for 24 h to remove germination inhibitors. Seed germination was performed using germination trays in a greenhouse in March 2011. Three weeks later, two-leaf seedlings transferred to the field and each of genotypes cultivated in two rows spacing 30 cm in a plot 3 m long. Fertilizers were applied at sowing (25 kg N and 30 kg P) and heading stages (25 kg N). Weeding was conducted manually during all growth stages. Irrigating plants was performed every 6-7 days as it was needed.

Table 1. *Plantago ovata* genotypes collected from different parts of Iran

Genotype	Origin	Latitude
Abadeh	Abadeh	31.30° N, 53.11° E
Darab	Darab	28.74° N, 54.55° E
Fasa	Fasa	28.93° N, 53.64° E
Firuzabad	Firuzabad	28.85° N, 52.53° E
Jahrom	Jahrom	28.95° N, 53.950° E
Kazerun	Kazerun	26.72° N, 54.28° E
Marvdasht	Marvdasht	29.80° N, 52.83° E

Leaf area (LA) (cm^2) and plant height (PH) (cm) were measured by selecting 10 plants in each plot during growing season. 0.5 g leaf samples from randomly selected plants used for quantifying chlorophyll (Chl) *a* and *b* contents by a spectrophotometer instrument based on a procedure proposed by Lichtenthaler and Wellburn [6] and using the following equations:

Chl a= 12.25 A663- 2.79 A646
Chl b= 21.21 A646- 5.1 A663

Where, A663 and A646 denote absorbance at 663 and 646 nm wave lengths, respectively. Enzymatic antioxidants (U g^{-1} leaf fresh weight (LFW)) consisting of ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT) were spcectrophotometrically read by using Nakado and Assad [28], Beauchamp and Fridovich [4] and

Dhindsa et al. [23] procedures, respectively. Spike length (SP) (cm), seed number per spike (SNS), seed number per plant (SNP), spike number per plant (SP) and grain yield (g) per plant (GY) were measured after harvesting 10 plants in each plot.

The seed swelling factor (SSF) (ml) was determined by soaking 1 g seed in 25 ml distilled water for 24 h [21]. The mucilage (g) content of seed was obtained by initially boiling of 1 g seed in 10 ml chloridric acid (1%) until the complete release of mucilage into the water [20, 26]. 60 ml of ethyl alcohol (96%) was added to the initially isolated mucilage extracts. The mucilage precipitates were then dried in oven for 22 h at 54 °C and powdered. The mucilage swelling factor (MSF) was also calculated by the following equation [15]:

Swelling factor of 1 g mucilage (ml g^{-1}) = (seed swelling factor × 100)/ mucilage content

Statistical analyses
Data from all characters were subjected to the analysis of variance (ANOVA) and the means were compared using the least significant differences (LSD) test in SAS software (SAS Institute, V. 9.3. 2011). Phenotypic (σ_p^2)and genetic (σ_g^2) variances were estimated from the error (σ_e^2) and genotype ($\sigma_e^2 + r\sigma_g^2$) expected mean squares in ANOVA for randomized complete blocks design. Phenotypic (CV_P) and genetic (CV_g) coefficients of variation were calculated using $\frac{\sqrt{\sigma_p^2}}{\bar{X}}$ and $\frac{\sqrt{\sigma_g^2}}{\bar{X}}$, respectively, where $\bar{X}$ is the mean of trait. Heritability (h^2) estimates were also calculated based on the following formula [27]:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

Clustering genotypes for the traits of interest was conducted based on similarity matrix and complete linkage [22] by using Statistica V. 7 software.

RESULTS

Anova and variations in traits means
The mean of squares for the effect of genotype were significant for all traits except MW and MSF indicating variations among *Plantago* genotypes (Table 2). LA varied from 275 cm^2 in the accessions originated from Jahrom to 781.6 cm^2 in Marvdasht (Table 2). The highest plant height was observed in Marvdasht (35.3 cm), Fasa (35.0 cm) and Abadeh (34.5 cm) while Darab (29.2 cm) was the shortest. The accessions Firuzabad (11.8 mg g^{-1} LFW) and Jahrom (8.5 mg g^{-1} LFW) had significantly higher Chl *a* and *b* than other accessions respectively. Mean comparison for enzymatic antioxidants indicated that the highest APX (43.6 U g^{-1} LFW), CAT (0.24 U g^{-1} LFW) and SOD (336 U g^{-1} LFW) quantified in Fasa, Darab and Jahrom, respectively (Table 2). The lowest antioxidant activity belonged to the accessions Firuzabad (36.3 and 74.2 U g^{-1} LFW for APX and SOD) and Kazerun (0.05 U g^{-1} LFW for CAT).

P. ovata accessions were not significantly different for mucilage weight. MW averaged 0.14 g in *Plantago* accessions. Although the mean of square for MW was not significant, it ranged from 0.10 g in Darab to 0.15 g in Abadeh and Firuzabad. *Plantago* accessions significantly differed in seed swelling factor. SSF, averaged 11 ml, was in the range of 8.2 to 16.7 ml in the wild accessions originated from Kazerun and Abadeh, respectively. After the accession Abadeh, Jahrom (11.7 ml) and Marvdasht (11.2 ml) were in the second and third ranks for SSF. There were no significant differences among *Plantago* accessions for MSF that averaged 7894.4 ml g^{-1}, although MSF varied between 7026 to 12873 ml g^{-1} in Marvdasht and Abadeh respectively (Table 2).

Grain yield components showed great variations among *Plantago* accessions. Average SL ranged from 2.8 to 7.5 cm in Abadeh and Kazerun respectively (Table 2).

Table 2. Mean comparison, ANOVA results and genetic variation parameters in *Plantago ovata* genotypes for mucilage quality, morphological traits and grain yield.

Genotype	LA	PH	Ch a	Chb	APX	CAT	SOD	MW	SSF	MSF	SL	SNS	SNP	SP	GW	GY
Firuzabad	333.9	30.6	11.8	8.0	36.3	0.14	74.2	0.15	9.9	7118	3.1	116	2797	38.3	5.3	61.9
Kazerun	359.8	33.3	9.5	6.5	38.7	0.05	213.7	0.12	8.2	7283	7.5	179	3726	46.3	6.1	124.1
Marvdasht	781.6	35.3	5.5	3.7	39.5	0.12	227.7	0.16	11.2	7026	3.2	168	5080	113.3	7.8	83.7
Darab	703.8	29.2	5.8	4.4	41.9	0.24	239.0	0.10	9.03	8697	3.1	215	2670	111.3	4.5	52.1
Jahrom	275.0	31.3	9.9	8.5	37.7	0.18	336.7	0.13	11.7	9049	4.0	185	1718	111.6	3.0	49.6
Abadeh	391.1	34.5	6.2	4.2	39.4	0.15	238.5	0.15	16.77	12873	2.8	152	2092	34.0	3.8	70.5
Fasa	336.8	35.0	6.0	3.6	43.6	0.07	246.0	0.13	10.6	9061	3.1	270	2378	105.0	3.6	54.0
Mean±	454.5±	32.8±	7.8±	5.6±	39.62±	0.14±	225.1±	0.14±	11.0±	8728±	3.8±	183.8±	2923±	71.4±	4.9±	70.89±
SEM	76.0	0.90	0.95	0.78	0.93	0.024	29.3	0.007	1.0	771.0	0.62	18.4	431.4	14.4	0.63	10.0
LSD (1%)	206.6	4.6	1.7	1.3	5.7	0.04	59.7	0.12	3.8	7894.4	1.1	38	1196	51.4	2.0	25.1
ANOVA																
Genotype[a] mean squares	121354	17.2	19.1	12.7	518.2	0.013	18095	0.0038[ns]	23.4	1250081[ns]	8.3	7112	3906250	4013	8.4	2083
Error mean squares	6867	3.48	0.51	0.27	5.3	0.0003	574.4	0.0025	2.4	10020578	0.2	240	230089	65	0.69	101.8
Genetic parameters																
σ_g^2	38162	4.57	6.23	4.17	4.3	0.00412	5840	0.00043	7.0	826764	2.7	2290	1225387	1316	2.5	660
σ_p^2	40451	5.73	6.4	4.26	6.0	0.00423	6031	0.00126	7.8	4166957	2.78	2370	1302084	1337	2.8	694
PCV (%)	42.2	7.31	32.13	37.0	6.2	47.6	34.5	26.1	25.2	23.4	43.4	26.5	39.0	51.2	34.3	37
GCV (%)	43.0	6.52	31.7	36.5	5.2	47.0	34.0	15.3	24.0	10.42	43.0	26.0	37.8	50.7	32.9	36
h^2 (%)	94.0	79.0	97.0	98.0	70.7	97.4	97.0	34.2	89.7	20.0	97.6	96.6	94.1	98.4	92.0	95

a: except Car, MW and MSF, genotype mean squares were significant at 0.01 probability, SEM: standard error of the mean, LA: leaf area (cm^2), PH: plant height (cm), Ch: chlorophyll (mg g^{-1} leaf fresh weight), APX: ascorbate peroxidase (U g^{-1} leaf fresh weight), CAT: catalase (U g^{-1} leaf fresh weight), SOD: superoxide dismutase (U g^{-1} leaf fresh weight), MW: mucilage weight (g), SSF: seed swelling factor (ml), MSF: mucilage swelling factor (ml g^{-1}), SL: spike length (cm), SNS: seed number per spike, SNP: seen number per plant, SP: spike per plant, GW: grain weight (g) per plant, GY grain yield (g) per square meter, σ_g^2: genetic variance, σ_p^2: phenotypic variance, PCV: phenotypic coefficient of variation, GCV: genetic coefficient of variation, h^2: heritability

The accession Jahrom (4.0 cm) was in the second rank having the highest SL after Kazerun. Spike length is one of the important features in discriminating different plant species in taxonomy. *P. ovata* accessions had great variations for SNS ranged from 116 in Firuzabad to 270 in Fasa significantly. Jahrom (185), Darab (215) and Fasa (270) were above the average (183.8) of seven *Plantago* accessions for SNS. Although SNP averaged 2923, Marvdasht having 5080 seeds, was in the first rank among *Plantago* accessions. There was no significant differences among Darab, Firuzabad and Kazerun with 2670, 2797 and 3726 seeds per plant. The lowest SNP (1718) belonged to Jahrom which was nearly threefold less than SNP in Marvdasht accession. SP ranging from 34 to 113.2 averaged 71.4 in *Plantago* accessions. Marvdasht, Jahrom, Darab and Fasa with 113.3, 111.6, 111.3 and 105 differed significantly form Abadeh, Firuzabad and Kazerun with 34, 38.3 and 46.3 for SP. End use grain yield was significantly differed in *Plantago* accessions (Table 2). GY with the average of 4.9 g ranged from 3 to 7.8 g. Marvdasht (7.8 g) and Kazerun (6.1 g) were significantly differed from Jahrom (3 g) for GY. The accessions Marvdasht, Kazerun and Firuzabad were above the average GY of all genotypes. Abadeh, Jahrom and Fasa, non-significant statistically, had the lowest GY among *P. ovata* accessions.

Genetic variation and heritability

Genetic variation parameters and heritability estimates of the traits are given in Table 2. Estimated genetic and phenotypic variances indicated that there were great differences between these variances for MW and MSF. This shows higher contribution of environmental effects in phenotypic variations of MW and MSF compared to other traits. The PCV and GCV of the morphological traits differed, as did the PCV and GCV for antioxidants, mucilage parameters and grain yield components. For instance, among morphological traits, PH had lower PCV (7.31%) and GCV (6.52%) than LA (43% and 43.2%) and SL (43.6% and 43%). The lowest PCV (6.2%) and GCV (5.2%) were obtained for APX. Genetically, CAT was very variable between *Plantago* accessions with GCV of 47%. Among mucilage parameters, SSF (GCV= 24%) was more variable than MW (GCV= 15.3%) and MSF (GCV= 10.4%). Among grain yield components, the highest GCV calculated for SNP (37.8%) and SP (50%) while SNS (26%) had the lowest GCV.

The heritabilities for morphological traits differed, as did the heritabilities for mucilage parameters. SL (97.6%) and LA (94%) are highly heritable compared to PH (79%). Estimated heritabilities for mucilage parameters indicated that MSF (20%) and MW (34.2%) are less heritable than SSF (89.7%). The heritabilities estimated for SOD (97%) and CAT (97.4%) were considerably higher than that estimated for APX (70.7%). Grain yield and its components were highly heritable with heritability values above 90%. SP had the highest (98.4%) heritability among grain yield components.

Correlation analysis and clustering *plantago* accessions

The correlation matrix of the traits is available in Table 3. LA was significantly correlated with GY (r= 0.62), SNP (r= 0.67) and SP (r= 0.84). Chl *a* and *b* were highly (r= 0.95) correlated although they had negative and significant relation with APX. Antioxidants showed no and/or negative correlations with GY and grain yield components. Both SSF and MSF, highly (r= 0.86) correlated mucilage parameters, had negative associations with GY. Although non-significant, the correlation coefficient (0.52) of MW and SSF was positive. Grain yield had significant and/or positive relations with SP (r= 0.33), SNP (r= 0.98) and SL (r= 0.80).

Table 3. Correlation coefficients between traits pair in *Plantago ovata* genotypes.

Trait	LA	PH	Chl*a*	Chl*b*	APX	CAT	SOD	MW	SSF	MSF	SL	SNS	SNP	SP	GW	GY
LA	1.0															
PH	0.02	1.0														
Ch *a*	-0.64	-0.45	1.0													
Ch *b*	-0.60	-0.55	0.95	1.0												
APX	0.31	0.26	-0.80	-0.80	1.0											
CAT	0.38	-0.69	-0.14	0.08	-0.03	1.0										
SOD	0.01	0.16	0.40	-0.13	0.36	0.19	1.0									
MW	0.02	0.46	0.12	0.05	-0.52	-0.16	-0.33	1.0								
SSF	-0.14	0.41	-0.31	-0.23	-0.06	0.16	0.25	0.52	1.0							
MSF	-0.24	0.20	-0.38	-0.30	0.22	0.24	0.37	0.03	0.86	1.0						
SL	-0.26	0.03	0.40	0.33	-0.23	-0.55	0.04	-0.36	-0.38	-0.38	1.0					
SNS	0.05	0.18	-0.81	-0.75	0.90	-0.12	0.54	0.64	0.06	0.06	-0.03	1.0				
SNP	0.67	0.38	-0.41	-0.38	0.31	0.21	-0.27	0.31	-0.62	-0.62	0.27	-0.17	1.0			
SP	0.84	0.17	-0.81	-0.75	0.38	0.42	0.11	0.09	0.35	0.31	-0.41	0.10	0.40	1.0		
GW	0.62	0.28	-0.09	-0.21	-0.20	-0.31	-0.42	0.36	-0.35	-0.63	0.29	-0.34	0.98	0.33	1.0	
GY	0.04	0.37	0.12	0.01	-0.23	-0.65	-0.18	0.02	-0.25	-0.30	0.83	0.24	0.62	0.02	0.65	1.0

**: coefficients higher than absolute values of 0.62 and 0.80 are significant at 0.05 and 0.01 probability levels, LA: leaf area (cm^2), PH: plant height (cm), Ch: chlorophyll (mg g^{-1} leaf fresh weight), APX: ascorbate peroxidase (U g^{-1} leaf fresh weight), CAT: catalase (U g^{-1} leaf fresh weight), SOD: superoxide dismutase (U g^{-1} leaf fresh weight), MW: mucilage weight (g), SSF: seed swelling factor (ml), MSF: mucilage swelling factor (ml g^{-1}), SL: spike length (cm), SNS: seed number per spike, SNP: seed number per plant, SP: spike per plant, GW: grain weight (g) per plant, GY grain yield (g) per square meter,* The cluster analysis of genotypes indicated that *P. ovata* accessions classified in four major groups (Fig. 1). Abadeh and Marvdash grouped in two single- member clusters that were distantly separated from other major groups. The third major group divided into a minor group consisting of the accessions Darab and Fasa with higher similarity than Jahrom as single minor group. Our results clearly indicated that Firuzabad and Kazerun closely joint in the fourth major group of *P. ovata* accessions.

DISCUSSION

Plantago is known to be relatively plastic for morphological and physiological characters. Phenotypic plasticity is the main mechanism by which *Plantago* species cope with different environments [7]. Landrace and wild accessions with enormous genetic variation and a reservoir of genetic diversity constitute a wide germplasm harboring unique genes and characters to breed for biotic and abiotic stresses conditions and adaptability to the various environments [10].

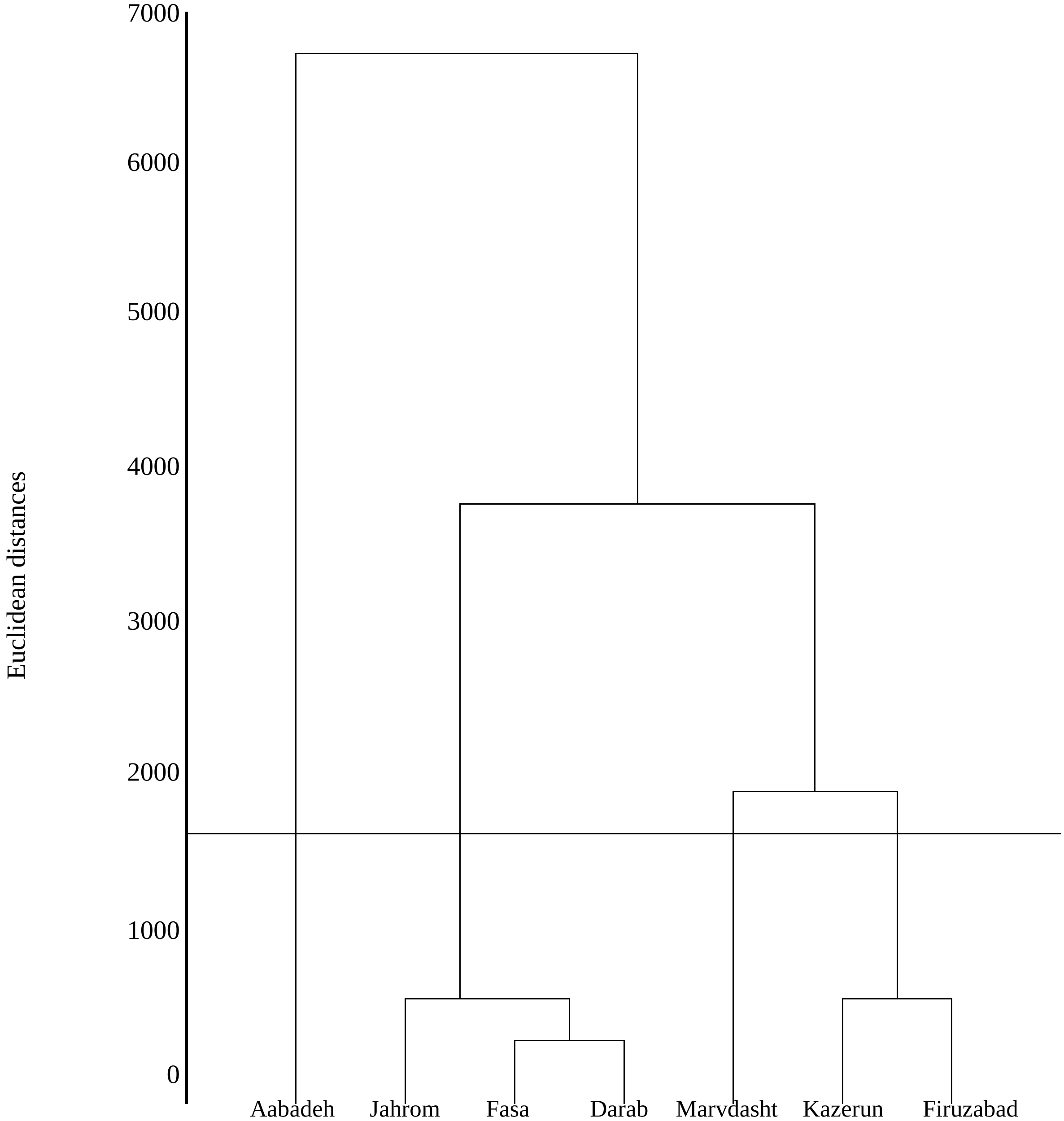

Figure 1. Dendrogram of different clusters of wild *P. ovata* accessions collected from different ecological habitats of Iran using their similarities in grain yield, mucilage and morphological traits.

Given the industrial and medicinal uses of husk and seeds of *P. ovata*, a lot of attentions have been paid to develop varieties for higher yield and swelling factor in these species [2, 3, 24]. Results of our study indicated that wild accessions of *P. ovata* are highly variable for morphological and grain yield components traits and also seed swelling factor. The mean mucilage of 1 g seed of *P. ovata* was 0.14 g while it was 0.08 g in the study reported by Vahabi et al. [3]. Although *Plantago* accessions in the present study were not differed for mucilage content, they showed significantly various seed swelling factor ranging from 8.2 to 16.77 ml. The accessions Marvdasht, Firuazabad and Abadeh with the highest mucilage content can be used in breeding programs targeting higher mucilage content. Das [15] by evaluating *P. indica* genotypes indicated that swelling factor varied from 9.6 to 12.3 ml which was lower than the range (18 to 22 ml) that was reported by Vahabi et al. [3] in *P. ovata*. In the study conducted on seed characteristics of Isabgol (*P. ovata* Forsk), seed swelling factors ranged from 12.67 to 14.61 ml depend on irrigation intervals [17].

Spike length is one of the important morphological features that can be used in classification of plant species. Morphologically, *P. ovata* accessions showed great variations in this study. The accession Abadeh had the lowest (2.8 cm) spike length that was significantly differed from Kazerun (7.5 cm) being the tallest accession. Evaluation of genetic variability in *Plantago* species indicated that the two ssp., *major* and *pleiosperma*, differ in growth form of leaves and inflorescences [7]. Our study revealed that Marvdasht had the highest plant height, mucilage, seed per plant, spike per plant and grain yield. Therefore, this accession can be considered as a candidate for increasing both grain yield and seed mucilage content. Abadeh had the highest seed and mucilage swelling factors but it showed the lowest spike length, seed number and spike among seven accessions. This result indicates that there is negative relation between swelling factor and grain yield in *P. ovata* that is in agreement with the results of Vahabi et al. [3]. Characterizing *Plantago* accessions for different morphological and mucilage characters helps breeders to identify the high and the low performing genotypes that can be used in hybridization programs.

High levels of genetic variations provide ample raw materials for plant breeding programs. Genetic coefficient of variation estimates indicated that *P. ovata* accessions had the lowest genetic variations for plant height while wide variations were observed for seed swelling factor, number of spike per plant, spike length, seed number and grain yield. Genetic diversity gives species the ability to adapt to changing environments including new pests and diseases and new climate conditions [10]. In present study, heritability estimates were relatively low for mucilage content and its swelling factor that show the influence of environmental effects on phenotypic variation or limited genetic variation for these characters. Other characters including seed swelling factor, grain yield components and morphological traits were highly heritable. Therefore, they would efficiently respond to selection in breeding programs targeting higher seed swelling factor and high yielding varieties of *Plantago*. Wolff et al. [12] indicated that different *Plantago* species varied in amount of variation, heritability estimates and in environmental sensitivity.

Our results for correlation analysis indicated that breeding for higher grain yield of *Plantago* may results in lower swelling and mucilage as they were negatively correlated. Allocating assimilates to higher grain yield reduces reservoirs for mucilage accumulation. Mucilage content was strongly correlated with seed number per spike. Therefore, selection for higher seeded genotypes would increase mucilage content in the seed of *Plantago*. Antioxidants are the first line defense system against stresses that highly accumulated under both biotic and abiotic conditions, therefore normal growth conditions of the present work may be a reason for the negative correlations of enzymatic antioxidants and grain yield. According to strong correlations, it can be concluded that higher grain yield was due to high leaf area and spike per plant, therefore selection strategies based on these traits would be efficient in breeding for high yielding *Plantago* varieties. Our results revealed that seven *P. ovata* accessions classified into three major groups based on their similarities in cluster analysis. Wild accessions originated from Jahrom, Fasa and Darab formed a major group. They had no significant differences in mucilage, seed swelling factor, spike length, grain weight and grain yield. The regions that these accessions originated from have relatively warm and similar climates. The accessions originated from Abadeh with cold semi-arid steppe climate and from Marvdasht with temperate climate were separately clustered in two major groups. These accessions were significantly different in seed swelling factor, spike and seed number and also grain yield. Firuzabad and Kazerun that joint as a major group were statistically similar in plant height, seed swelling, seed and spike number per plant, grain yield although they differed from seed number per spike and spike length. This results show that *P. ovata* accessions benefit from different characters for their ecological adaptability to the environments.

CONCLUSION

In conclusion, evaluation of *P. ovata* accessions indicated that wild genotypes are valuable ample raw materials that are highly variable for grain yield, morphological traits and seed swelling factor and that they differed from each other depending their ecological habitats. Therefore, identification of different wild accessions provides useful information for breeding *P. ovata* varieties for various purposes.

REFERENCES

[1] AK Koul; S Sareen, *Plantago ovata* Forsk: Cultivation, Botany, Utilization and Improvement. In: "Supplement to Cultivation of Medicinal Plants". Eds.: Hand SS, Kaul M K, Regional Research Laboratory Council of Scientific and Industrial Research, Jammu-Tawi, India., **1999**, Pp. 477-495.
[2] AK Rohilla; M Kumar; M Sindhu; KS Boora, *African Journal of Biotechnology.*, **2012**, 92, 15835-15842.
[3] AAVahabi; A Lotfi; M Solouki; S Bahrami, *Biotechnology.*, **2008**, 7, 702-709.
[4] C Beauchamp, I Fridovich, *Annals of Biochemistry*., **1971**, 44, 276-287.
[5] F Dawidowsky. Glue, Gelatin, Animal Charcoal, Phosphorus, Cements, Pastes, and Mucilage. BiblioLife. **2009**, pp. 1.
[6] H Lichtenthaler; AR Wellburn, *Biochemistry Society Transaction*, **1983**, 603, 591-592.
[7] H Van Dijk, *Theoretical and Applied Genetics.*, **1984**, 68, 43-52.
[8] H Van Dijk, *Theoretical and Applied Genetics*., **1989**, 77, 749-759.
[9] I Beara; DZ Orici; MM Lesjak; NM Mimica-Dukic; BA Pekovic; MR Popovic, *Journal of Pharmaceutical and Biomedical Analysis.*, **2010**, 52, 701-706.
[10] K Hammer; Y Teklu, *Journal of Agriculture and Rural Development in Tropics and Subtropics*, **2008**, 109, 15-50.
[11] K Rahn, *Botanical Journal of the Linnean Society*, **1996**, 120,145-198.
[12] K Wolff; WV Deldan, *Heredity*, **1987**, 58, 183-192.
[13] K Wolff; M Morgan –Richards; AW Davison, *New Phyto*., **2000**, 145, 501-509.
[14] KC Dalal; S Sriram, Psyllium. In: Advances in Horticultural Medicinal and Aromatic Plants. (Eds. Chaddah, K. L., Gupta, R.), Malhotra Publishing House, New Delhi, India, **1995**, 2, 575-604.
[15] M Das, *International Journal of Plant Physiology and Biochemistry., **2011***, 3, 205-214.
[16] MK Dhar; S Kaul; S Sareen; AK Koul, *Plant Genet. Resour. Cultivat. Utilizati.*, **2005**, 3, 252-263.
[17] M Mohebi; A Maleki, *Advances in Environmental Biology*., **2010**, 4, 10-13.
[18] M Saeedi; K Morteza-Semnani; S Fallahi; G Amin, *Acta Pharmacology*, **2010**, 60, 339-348.
[19] MK Dhar; B Friebe; S Kaul; B Gili, *Annals of Botany.*, **2006**, 97, 541-548.
[20] NK Kalyanasundaram; S Sriram; BR Patel; RB Patel; DH Patel; KC Dalal; R Gupta, *Indian J. Horticulture.*, **1984**, 28: 35-37.
[21] PK Sharma; AK Koul, *Journal of Ethnopharmacology*., **1986**, 17, 289-295.
[22] RA Johnson, DW Wichern, Applied multivariate statistical analysis. Pearson: sixth Edition, **2007**, 800 pp.
[23] RS, Dhnidsa; P Plumb-Dhindsa; TA Thorpe, *Journal of Experimental Botany* **1981**, 32: 93-101.
[24] S Saeedi; F. Munir; I. Naveed; GK Raja; T Mahmood, *Journal of Medicinal and Plants Research.*, **2011**, 5, 4888-4891.
[25] S Samantaray; UM Dhagat; S Maiti, *Plant Biotechnology.*, **2010**, 27, 297-303.
[26] SP Washi; VD Sharma; VK Jain; P Sinha, *Indian Journal of Nat. Prod.* **1985**, 1, 3–6.
[27] WR Fehr, Applied Plant Breeding, Iowa State University, Second Edition. **1983**
[28] Y Nakano, K Asada, *Plant Cell Physiology.*, **1981**, 22, 867-880.