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Morphological and Phytochemical Study of Indigenous *Plantago Ovata* of Golestan Province in Iran

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ABSTRACT

Isabgol (Plantago ovata) belonging to Plantaginaceae family is a medicinal plant useful as a laxative, and blood cholesterol, fat and sugar reducer. Lots of these plants grow wildly in some parts of Golestan province. An appropriate method for specifying the plant's selection criteria is gathering its indigenous species and evaluating the morphological characteristics that determine its yield. The present study was conducted to investigate the morphological and phytochemical characteristics of different Isabgol plants under Azadshahr weather conditions. To this end, plant seeds from four different habitats (Incheboron, Duzavlom, Gharamakher and Aramgah) were gathered and cultivated in the form of completely randomized blocks with 4 replications during March, 2011. The traits under evaluation were spike number and length, grain number per spike, plant height, grain weight, mucilage rate and percentage, inflation factor and inflation rate per gram of mucilage. The obtained data were analyzed applying SPSS software. Analysis of variance indicated no significant differences among the investigated plants in terms of plant height, spike number per plant and inflation factor. However, spike length, grain numbers per spike and 1000 seeds weight were significantly different among the plants at.05 level of significance. Moreover, plant seeds were significantly different in terms of mucilage rate, mucilage percentage, and inflation rate per gram of mucilage at.01 level of significance. The minimum means of spike lengths (9.16 mm) in Incheboron mass and the other three Plantago species were not significantly different. Comparison of the average grain numbers per spike also showed the highest grain numbers in Gharamakher (19.25 number) and the lowest spike numbers in Incheboron (15.61 number) and Duzavlom (15.95 number) plants. Besides, the highest and the lowest mucilage rates were related to Gharamakher (0.259 gr) and Incheboron (0.168 gr) species.

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INTRODUCTION

Compared to most of the areas of the world such as the European continent, Iran is one of the world's greatest treasures of aromatic and medicinal plants and also one of the eight genetic sources of medicinal plants. This is because the vast Iranian land has different territories with temperature variations of more than 50° C and possesses coastal, mountain and desert regions with at least 300 sunny days per year (12). Isabgol scientifically known as *Plantago ovata* is a medicinal plant being included in plantaginaceae family has narrow, long and sharp tip leaves covered with soft silver strings (1). Isabgol is an important economic crop in various parts of India, such as North Gujarat. India is the largest producer of Isabgol (12) and exporter of its seeds (4 and 15), and seed shells which are worth millions of rupees a year. This plant is grown in some parts of Iran for its economical advantages (12). The seeds and leaves of Psyllium contain Aucubin glycosides (10, 21 and 12), Tannin (12) and a carbohydrate gum known as Xyline (9). Psyllium is also full of glue-like compounds called mucilage (12, 10, 5 and 21). Shell powder of this plant was widely applied in traditional medicine as a laxative (1, 10, and 9). Research suggested that the fibers existing in Psyllium have significant influences on lowering blood cholesterol, fat and sugar in diabetic people and decreasing the risk of colon cancer (10). Classic methods for measuring varieties of different plant species have been based on morphological properties. During these studies the plants under investigation were assessed based on some of the morphological differences in their observable traits (11).

So far, many research studies have been conducted to assess genetic diversity based on morphological traits of medicinal plants (2). Analysis of genetic diversity is the basis for plant breeding programs. The plant breeder depends on the phenotypic data to detect the genetic relationships among the genotypes (18). Major medicinal plants that have been used in the past and are still applied in most parts of the world grow wildly. The effective ingredients of these plants were identified and their amounts were considered quantitatively and qualitatively. Then, these plants were domesticated and cultivated to be used in medicine and treatment (16). Using secondary metabolites for investigating the variation and classification of the plants have been well determined in a way that these changes can be used in classification of the plants (20). Thus, identification and domestication of the selected population of the plants containing high efficiency from the natural masses of a species can be an important and significant progress in gaining the required types of the plants for the industries, without spending much time and extra costs for performing the breeding programs (13).

In this regard, some research studies have been conducted. Makkizadeh-Tafti et al (2010) evaluated the plant and yield characteristics, and essence components of the ecotypes of Kermani thyme in Iranian natural habitats (in Raine and Sirch of Kerman, Yazd, Isfahan and Shahrood). They observed the highest yields in the ecotype of Raine of Kerman and claimed that Shahrood ecotype with 38 ingredients has the greatest composition in its essence (8). In addition, Rahimi (2007) examined 20 coriander bushes domestic to Iran and observed significant differences among the bushes in terms of their essence rates. It was also found that the essence yield in this plant was significantly influenced by seed source (14). The genetic diversities of 65 genotypes of *Plantago ovata* collected from different sources (56 genotypes from India, 2 from America, 2 from Pakistan and 1 from France, Hungary, Poland, Iran, and Bulgaria together with P. major and *P. lanceolata* as the other species) were analyzed quantitatively for 3 years in terms of 9 economic traits including spike length, dry inflorescence yield (without seed) per plant, seed yield per plant, seed shell yield per plant, days up to 50 % flowering, plant height, branch number per plant, spike number per plant, and inflorescence tail length (7).

In a research study, the mucilage rate, inflation factor and 100 seeds weight of the Plantago were measured at three locations. These features were determined for Baghe Giahshenasi (15.8 percent, 9.93 ml, 126.26 mgr), Mardabad (15.6 percent, 13.6 ml, 141.5 mgr) and Hoomand Absard (22.35 percent, 13.46 ml, and 170.8 mgr), respectively. The same characteristics were measured in Mashhad. The means of these characteristics were respectively 17.2 percent, 11.83 ml and 143.5 mgr (6). According to the discussed issues, identification and selection of the ecotype or species with prominent characteristics among the collected plants of Golestan Province can increase the qualitative and quantitative yields of the plant. The major portion of this crop is supplied by Indian markets. Thus, if the possibility for production of this plant is created, the incomes and lives of the farmers will be significantly promoted. Meanwhile, cultivation of the plant can bring economic revenues for the country through exporting.

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MATERIALS AND METHODS

The purpose of the present study was the morphological and phytochemical analysis of various Plantago plants under Azadshahr (located in Golestan province) weather conditions. To this end, growth locations of this plant were identified using library, flora and vegetation resources, and experts' experiences together with the aid of people native to the Golestan province. The seeds were collected on May 2011 from four regions (including Gharamakher, Duzavlom, Marta Aramgah, and Incheboron) where the plant grows wildly. The collected seeds were cultivated and studied in the form of randomized complete blocks design with 4 replications on March 2011 in Azadshahr, Golestan with eastern geographical longitude of 55° 14′ 58″ and northern geographical latitude of 37° 06′ 54″. Soil properties of the treatment site are summarized in Table 1.

Table 1. Soil Properties of the Treatment Site

Texture/Soil	%Sand	%Silt	%Clay	K(ava) p.p.m	P(ava) p.p.m	%Total N	%O.C	%T.N.V	PH of paste	EC×10³	S.P
Silt/clay	11	48	39	249	28.7	.2	2	5.21	7.6	1.81	60.4

The morphological characteristics under evaluation included spike number and length, grain number per spike, plant height, 1000 seeds weight, and phytochemical (qualitative) traits such as mucilage rate and percentage, inflation factor and inflation rate per each gram of mucilage.

In order to measure the mucilage, one gram of dry seeds was added to 10 ml and.1 normal boiling Hydrochloric Acid and heating was continued till the change of the color of the seed shells. Then, heating was stopped and the obtained mucilage solution was immediately filtered and separated. In order to filter the residual amounts of mucilage, the seeds that were still warm were washed twice with 5 ml boiling water. The resulting solution was filtered after each washing time and was added to the mucilage solution. In the following step, 60 ml Ethanol 96% was added to the filtered solution containing mucilage, well shaken, and transferred to the fridge for 5 hours to have mucilage sediments at the bottom of the dish. After this step, the solution was removed from the fridge. Then, its supernatant portion was discarded and the remained portions were filtered. The filter paper as the mucilage carrier was placed in an electric oven with air conditioning for 12 hours under the temperature of 50° C. Afterwards, the resulting sediments that were the representatives of the mucilage rate per gram of seed were weighted (17). The inflation rate for each gram of mucilage that can be used as the quality index of Plantago seeds was determined based on the following equation:

Inflation rate per gram= (mucilage inflation factor / mucilage rate) × 100

Statistical analyses including analysis of variance, comparison of the means and correlation of the traits were done using SPSS software. It should be noted that for comparing the means Duncan's Multiple Range test was used.

Results and Discussion

A - Analysis of Variance of Morphological and Phytochemical Traits

The results of the analysis of variance for traits of identified Plantago plants cultivated in crop conditions indicated significant differences in terms of mucilage rate, mucilage percentage, and inflation rate for each gram of mucilage at 0.01 level of significance. In addition, significant differences were observed in terms of spike length, grain number per spike and grain weight at.05 level of significance. However, the seed source on plant height, spike number and inflation factor were not significantly different (Table 2).

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Variables	df	Mean Squares								
		Plant height	Spike number	Spike length	grains Number per spike	1000 seeds Weight	Mucilage rate	Mucilage percentage	Inflation factor	Inflation rate per gram of mucilage
Replications	3	2.878 ^{ns}	7.280^{ns}	1.715 ^{ns}	8.715*	.018 ^{ns}	9.706 ×10 $^{-5\ ns}$	1.004^{ns}	.141^{ns}	8.153^{ns}
Locations	3	6.088 ^{ns}	22.628^{ns}	9.127*	11.354*	.061*	.007**	66.943**	.057^{ns}	732.568 **
Error	9	3.425	31.228	2.290	1.712	.016	.0002	1.769	.113	25.046
Coefficients of variation %		14.68	29.3	13.27	7.64	12.46	6	6.09	2.33	7.44

ns: not significant; *: significant at.05 level of significance; **: significant at.01 level of significance

B – Mean Comparison of Morphological and Phytochemical Traits under Investigation

Based on the mean comparisons (Table 2), the differences among the plants in terms of the plant height were not significant. However, the highest and the lowest plant heights were respectively related to Aramgah (14.03 cm) and Incheboron plants (11.39 cm). In addition, the highest and the lowest spike numbers were obtained from Duzavlom (13.18) and Incheboron plants (8.01), respectively. It should be noted however, that for spike number, there were no significant differences among the plants. Comparison of the means suggests that the maximum number of grains per spike is related to Gharamakher with 19.25 numbers of grains per spike. For 1000 seeds weight as the other trait, the highest and the lowest amounts were observed in Gharamakher (1.14 gr) and Duzavlom (0.88 gr), respectively. Mucilage rate whose maximum amount was observed in Gharamakher plants (0.259 gr), was not significantly different between Gharamakher and Aramgah plants. Besides, the lowest mucilage rate was obtained from Incheboron plants (0.168 gr) (Table 3). For inflation factor, there were no significant differences among the plants (Table 2), yet, the highest values of inflation factor were observed in Aramgah and Incheboron plants (Table 3).

Table 3. Mean Comparison of the Studied Traits in the 4 Cultivated Plants under Crop Conditions

Traits	**Plant Locations**			
	Aramgah	Incheboron	Duzavlom	Gharamakher
Plant height (cm)	14.035	11.39	11.77	13.20
Spike number per plant	12.2500	8.012	13.180	9.6000
Spike length (mm)	11.98^{a}	9.16^{b}	12.49^{a}	11.96^{a}
Grain number per spike	17.68^{ab}	15.61^{b}	15.95^{b}	19.25^{a}
1000 seeds Weight (gr)	1.087^{ab}	.937^{ab}	.885^{b}	1.147^{a}
Mucilage rate	.242^{a}	.168^{c}	.201^{b}	.259^{a}
Mucilage percentage	24.22^{a}	16.87^{c}	20.17^{b}	25.97^{a}
Inflation factor	14.5	14.37	14.5	14.25
Mucilage rate per gram of mucilage	59.97^{c}	85.40^{a}	71.97^{b}	55.09^{c}

Similar letters for each trait show non-significant differences

C - Pearson Correlations between Studied Traits in Plantago Plants of Golestan Province under Cultivation Conditions

Correlation coefficients for evaluated morphological traits (Table 4) indicated significantly positive correlations between plant height and 100 seeds weight at.01 level of significance and significantly positive correlations between plant height and spike number, plant height and spike length, and plant height and grain number per spike at.05 level of significance. Moreover, grain number per spike had significantly positive correlation with all the qualitative (phytochemical) traits except for inflation factor at.05 level of significance. Significantly negative correlation (-.545*) was observed between 1000 seeds weight and inflation rate for each gram of mucilage at.05 level of significance.

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Also, among the qualitative traits under investigation, mucilage rate showed significantly positive correlation with 1000 seeds weight (.516*) and grain numbers per spike (.536*) at.05 level of significance. An increase in 1000 seeds weight indicates that the grains will become heavier. Since 30% of the seed consists of mucilage, heavier grains that produce higher 1000 seeds weights will yield higher mucilage rates. Thus, the correlation between mucilage rate and 1000 seeds weight is justified. A perfect positive correlation (1.000 *) was observed between mucilage rate and mucilage percentage at.01 level of significance. Also, a very high negative correlation (-.984**) was observed between inflation rate per each gram of mucilage and mucilage rate at.01 level of significance. Additionally, inflation factor did not have any significant correlation with any of the qualitative and quantitative traits (Table 4).

Table 4. Pearson Correlation Coefficients between Studied Traits for 4 Plantago Plants of Golestan Province

	Inflation factor	Inflation per one gram of mucilage	Mucilage percentage	Mucilage rate	Weight of 1000 seeds	Number of grains per spike	Spike length	Spike number
Inflation per one gram of mucilage	.254							
Mucilage percentage	-.159	.984**						
Mucilage rate	-.158	-.984**	1.000*					
Weight of 1000 seeds	-.254	-.545*	.517*	.516*				
Number of grains per spike	-.089	-.518*	.535*	.536*	.562*			
Spike length	-.434	-.612*	.497	.497	.334	.326		
Spike number	.67	-.173	.127	.126	.307	.176	.395	
Plant height	-.156	-.489	.446	.447	.677**	.519*	.542*	.622*

* and **: significant respectively at.05 and.01 level of significance. Coefficients without star marks are not significant

According to the results of the morphological trait analysis, Gharamakher and Incheboron plants had respectively the highest and the lowest values for most of the traits. Furthermore, the highest values of phytochemical traits (mucilage rate and inflation factor) belonged to Gharamakher plants. In general, based on the obtained results of the present study, Plantago plants of Golestan province cultivated under crop condition had very high mucilage rates. Other research studies on Plantago plants suggested the existence of differences among the plants in terms of some of the traits. For example, Ebrahimzadeh et al (1996) evaluated Plantago plants of three locations including Baghe Giahshenasi, Mardabad, and Hoomand Absard in terms of three traits namely, mucilage percentage, inflation factor, and 100 seeds weight. They observed that these three traits differ in different locations (6). In a study conducted in Jabalpur by Barfa et al (2011), 16 germplasm *of Plantago ovata* were compared in terms of their protein percentage, inflation rate, fat percentage, crude fibre (in percent) and seed shells. Based on the results of this study, among 16 germplasm under investigation, Gujrat-1 was selected as the best germplasm (3). Besides, in a research study for evaluating the molecular variety and morphology of Plantago, 22 populations of this plant from different regions of Iran were collected and analyzed. In this study, quantitative traits including spike length, spike number per plant, grain number per spike, spike weight, plant height, straw yield and seed weight were measured together with qualitative traits including grain mucilage percentage, grain inflation factor and grain yield. The results of this analysis suggested that the highest and the lowest yields can be respectively observed in Isfahan (165.15 kg/ha) and Bandarabbas (494.15 kg/ha). The highest and the lowest plant heights were respectively observed in the collected plants from Karaj and Yazd. Moreover, the maximum and minimum numbers of spikes per plant were respectively observed in plants from Yazd and Zabol. In general, there were significant differences between all the measured traits except for 1000 seeds weight (19). All in all, based on the obtained results of the current study, it can be concluded that Plantago plants of Golestan province have a very high mucilage yield and Gharemakher plants are dominant in terms of all the traits that can be used for breeding programs.

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