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Full Length Research Paper

Study of some agronomic attributes in milk thistle (*Silybum marianum* Gaertn.) ecotypes from Iran

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To study agronomic attributes of milk thistle (*Silybum marianum* Gaertn.), an important medicinal plant, twenty-five milk thistle genotypes including twenty-three ecotypes from Iran along two introduced varieties, CN seeds and Budakalaszi, were grown in a randomized complete block design with three replications. Analysis of variance for studied attributes showed large variation among the ecotypes. Means of some ecotypes such as Tatar and Parsabad were the highest for seed yield in comparing to other ecotypes even the introduced breeding varieties. In regard to phenotypic correlations, seed number in capsule, seed weight in capsule and main capsule diameter were the most important milk thistle yield components. Cluster analysis based on studied traits categorized the entries into three distinct clusters. The grouping had some correspondence to the geographical pattern.

Key words: Cluster analysis, ecotype, milk thistle, yield components.

INTRODUCTION

Medicinal plants are supplied from wild and cultivation. Most of trading plant materials (local or international) are still harvested from wild resources and just few number of the plant species are cultivated (FAO, 2005). This led to overthrow plant genetic resources in developing countries and lack of genetic variation of medicinal plants. In attention to continuously supply of homogenous medicinal plants regarding to market requirement and fast decrease in plant resources, development of cultivated medicinal species may be an alternative way to overcome the problems. Some countries such as Argentina, China, Hungary, India, Poland and Spain are trying to develop cultivation of some medicinal plants at large scale (FAO, 2005). Milk thistle (*Silybum marianum* L.), is an important medicinal plant of the composite family, have valuable constitutes, flavonolignans, e.g. silybin, silychristin and silydianin that all called silymarin and are stored in achens (Morazzoni and Bombardelli, 1995). The flavonolignans are anti-hepatoxic activity and protect liver against toxin agents. Milk thistle sale has reached to over 8 million dollars in USA at 2005 (Blumenthal et al., 2006) and obtained 10th sale rank among medicinal plants at 2007 (Anonymous, 2010). At United States, acute liver disease is caused to more than 25000 deaths annually and has 10th rank as death reasons (Jacobs et al., 2002). Geneva et al. (2008) studied influence of different fertilizers on physiological parameters, generative and vegetative growth, seed yield and its components in milk thistle. Their results displayed positive relations between studied treatments and number of minor branches and number of capsule per plant. Also, changes of flowering rate and seed maturity with increasing seed yield and pharmaceutical constituents were caused by increasing levels of the treatments. Sulas et al. (2008) assessed biomass production in milk thistle under low input conditions without irrigation, fertilization and other agronomic inputs and stated that the plant may be appropriate alternative to high input plants in respect to water and nutrition elements usage at water restricted conditions. Ram et al. (2005) evaluated 15 milk thistle accessions including 5 local populations collected from Jammu's India along to 10 foreign populations collected from countries of Italy, Israel, Romania, Germany, Hungary and Argentina for morphological traits and silymarin. The maximum genotypic coefficient variations, heritability and genetic advance obtained for traits of seed yield in plant and

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Table 1. The description of the ecotypes studied in the experiment.

	Province	Accession number	Name	Altitude (m)	Latitude	Longitude
North of Iran	Ardabil	1	Parsabad	30	39° 38' N	47° 55' E
		2	Gharaghieh	240	38° 30' N	47° 46' E
		3	Babak	60	39° 26' N	48° 09' E
		4	Bilesavar	100	39° 21' N	48° 13' E
		5	Rouhkandi	30	39° 24' N	48° 13' E
		6	Anjirlou	150	39° 10' N	48° 07' E
	East Azarbaijan	7	Tatar	320	39° 30' N	46° 58' E
		8	Gharachilar	420	38° 52' N	46° 32' E
	Golestan	9	Gorgan	200	36° 50' N	54° 25' E
		10	Naharkhoran	420	36° 44' N	54° 28' E
	Mazandaran	11	Behshahr	90	36° 41' N	53° 32' E
		12	Nour	30	36° 34' N	51° 57' E
		13	Mahmoudabad	20	36° 37' N	52° 14' E
		14	Fereydounkenar	40	36° 40' N	52° 31' E
South of Iran	Khouzestan	15	Dezful	180	32° 22' N	48° 23' E
		16	Andimeshk	200	32° 27' N	48° 20' E
		17	Hamidieh	0	31° 28' N	48° 26' E
		18	Behbahan	300	30° 35' N	50° 14' E
		19	Shoush	85	32° 11' N	48° 14' E
		20	Ramhormoz	90	31° 16' N	49° 36' E
	Boushehr	21	Abpakhsh	20	29° 20' N	51° 04' E
	Lorestan	22	Jolge Khalaj	780	33° 17' N	47° 48' E
		23	Ghaemieh	800	29° 36' N	51° 39' E

number of capsule per plant. They found significantly positive correlations among number of capsule per plant, number of branches per plant and leaf length and also among seed yield, leaf length, stem diameter, capsule diameter and silymarin content.

Plant genotype and climatic status of habitats may play significant role on quantity and quality of medicinal constituents of plants. Milk thistle is grown in a wide range of climates and geographical areas in Iran. The first step in breeding of wild or native populations is collection and description of genetic variation of plant populations for desired characters. To study genetic variation in different plant species, agronomic attributes are often characterized. Since, environmental conditions of cultivation area may significantly affect morphological characters particularly seed yield. Therefore, this research was run to assess agronomic attributes of some milk thistle ecotypes from Iran.

MATERIALS AND METHODS

Twenty five genotypes used in this research consisted of twenty three ecotypes collected from different parts of Iran (Table 1) and two introduced varieties, Budakalaszi (from Hungary) and CNseeds (from England). In the month of April, 2008, these were grown in an experimental field of the Research Station of Faculty of Agriculture in Moghan, University of Mohaghegh Ardabili. The experiment was run based on a design of randomized complete block in three replications. In respect to results of previous studies about plant density (Foldesi and Szasz-Barsi, 1983; Omer et al., 1993), distance of among rows and among plants was made as 70 and 25 centimeters, respectively. Recommended agronomic practices were adopted (Omer et al., 1993; Hammouda et al., 1994). Data were recorded on the selected plants in each replication for seven traits (Table 2). Before analyzing data, Kolmogrov-Smirnov normality test of errors was performed (Steel and Torrie, 1980). The distribution was normal for all the traits. After univariate analysis, comparisons of means and bivariate phenotypic correlations, cluster analysis was done for classifying the entries. All statistical analyses were conducted by SPSS 16 software.

RESULTS

The analysis of variance of the data appeared significant differences among the ecotypes for all the studied traits (data not shown). In other words, there was a good level of phenotypic variation among the ecotypes. Coefficient of variations for the all traits obtained lower than 30% that presented the proper precision of the measuring process. The capsule diameter and number of capsule per plant had the least (6%) and the highest (30%) CVs, respectively.

Mean comparisons for 1000 seed weight and seed

Table 2. Means of agronomic attributes in milk thistle accessions.

Genotype	Capsule no./plant	1000 seed w. (gr)	Height (cm)	Capsule Diam. (cm)	Yield (kgha^{-1})	Seed w./cap. (gr)	Seed no./cap.
Behshahr	14.845	17.050	154.105	4.108	1315.452	1.281	76.271
Fereydounkenar	14.149	19.550	142.361	4.046	1574.360	1.809	95.890
Naharkhoran	15.329	17.625	138.192	4.228	1433.574	1.448	84.887
Budakalaszi	13.266	18.814	160.612	4.422	1509.999	1.763	92.647
Cnseeds	16.221	22.543	146.790	4.270	1648.127	1.783	80.704
Tatar	26.421	15.602	149.830	4.414	2416.673	1.406	91.737
Bilesavar	18.813	15.198	147.133	4.388	1666.619	1.425	95.424
Mahmoudabad	12.361	19.171	142.806	4.212	1337.222	1.680	91.396
Parsabad	23.449	16.704	146.353	4.346	2239.700	1.483	89.517
Dezfoul	11.854	22.736	133.040	4.009	1493.645	1.899	85.927
Nour	13.346	16.714	133.911	3.967	1197.599	1.462	88.651
Gharachilar	18.207	16.764	134.575	4.141	1674.417	1.357	81.016
Gorgan	16.705	16.550	150.285	4.406	1662.159	1.660	101.407
Gharaghieh	21.581	15.131	166.475	4.473	1785.651	1.224	79.527
Anjirloo	19.772	18.000	137.900	3.999	1617.761	1.287	73.055
Roohkandi	21.019	16.683	137.381	4.210	1652.565	1.183	71.288
Babak	19.537	15.465	146.667	4.404	1290.011	1.065	71.666
Shoush	21.800	19.227	141.400	3.857	889.583	1.042	56.123
Behbahan	24.800	19.457	139.867	3.955	999.174	1.014	53.068
Abpakhsh	22.967	21.703	129.267	4.130	1018.696	1.196	55.534
Jolgeh khalaj	23.333	19.680	159.022	4.486	1088.329	1.400	75.212
Ghaemieh	24.533	19.520	133.800	4.290	1031.523	1.392	73.030
Ramhormoz	26.917	18.558	136.233	4.011	1868.546	1.619	84.861
Andimeshk	24.800	17.153	151.867	3.775	1017.084	1.141	69.910
Hamidieh	14.817	19.377	131.867	4.027	961.930	0.970	51.643
$LSD_{5\%}$	9.420	2.898	19.980	0.425	520.300	0.451	26.930

weight per capsule elucidated differences between the ecotypes as the highest belonged to CNseeds and Budakalaszi along to ecotypes of Dezful, Mahmoudabad and Fereydounkenar. Also, the least means for 1000 seed weight belonged to Hamidieh, Behbahan, Shoush and Babak and for seed weight per capsule, Gharaghieh, Bilesavar, Babak and Tatar (Table 2). Ecotypes of Tatar and Parsabad had the highest means of seed yield while minimum seed yield was observed in south ecotypes e.g. Hamidieh and Behbahan. Phenotypic correlations of the traits displayed significantly positive relations between seed yield and three yield components, seed number per capsule, seed weight per capsule and capsule diameter (Table 3). In spite of that, seed yield increasing was not along to elevating all the yield components which there was no significant association among seed yield and 1000 seed weight. The high yield ecotypes such as Tatar and Parsabad had also the largest values for the three said yield components. Consequently, these yield components may play important role in indirect selection for seed yield. Positive significant correlation was found for plant height and capsule diameter. That is, ecotypes with the more plant height have the larger capsules, too.

Classifying the entries for the measured data was done by WARD's cluster analysis (Figure 1). On this basis, the most ecotypes of the south parts located in one group (first cluster). However, in the second cluster, three ecotypes of Nour, Fereydounkenar and Mahmoudabad from North of Iran, Dezful from south and variety of CNseeds were existed. The remained entries included all from west north of Iran, Budakalaszi variety and other ecotypes from different parts of Iran located in third cluster. To determine the clusters properties, mean deviations from those of total means of the clusters were computed for all the studied traits (Figure 2). The ecotypes of first cluster (Southern ecotypes) had means of 1000 seed weight and capsule no. per plant more significantly than total means. In spite of that, they were significantly lower for other traits particularly seed yield (-32%). In other words, this cluster may be characterized in respect to low yield. In attention to large climatic differences among the ecotypes habitats and experiment location, their weak phenotypic performance may be caused by their non-convenient adaptation. The most of north ecotypes along to CNseeds were grouped into the second cluster, had lower plant height, seed yield,

Table 3. Correlation coefficient among agronomic attributes in milk thistle accessions.

	Cap. no./plant	1000 seed w.	Height	Cap. diameter	Yield	Seed w./cap.
1000 seed w. (gr)	-0.20					
Height (cm)	0.06	-0.38				
capti. Diam. (cm)	0.02	-0.32	0.54**			
Yield (kg ha^{-1})	0.12	-0.40*	0.24	0.46*		
seed w./cap. (gr)	-0.47*	0.33	0.08	0.23	0.43*	
seed no/capt	-0.35	-0.30	0.32	0.45*	0.66**	0.80**

* and ** significant at the 0.05 and 0.01 level, respectively.

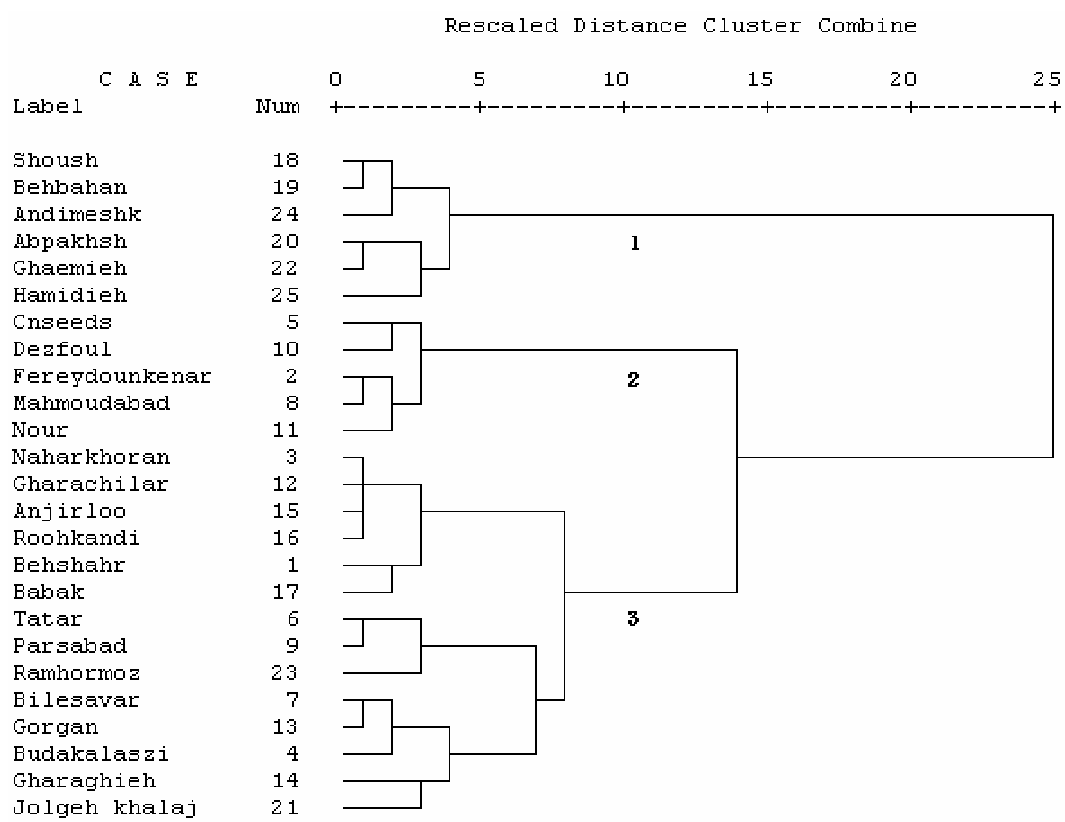

Figure 1. Classification of milk thistle accessions by ward's cluster analysis for morphological traits.

capsule diameter and capsule no. than total means of those. They were superior for remained traits. Totally, in this cluster, means of the most attributes except to seed no. per capsule and seed weight per capsule (yield components), had no significant difference to those of total means. Amounts of deviation from total mean for seed yield (-5.4%) and some yield components e.g. capsule number (-5.6%) showed that the ecotypes of the cluster may be known as intermediate yield group.

The third cluster included all North West ecotypes and some other ecotypes along to variety of Budakalaszi. The values of mean deviations of the attributes from this group were unlike to the second cluster. That is, except to 1000 seed w. and seed w. per capsule, they were higher than total means for remained traits; chiefly seed yield (14% higher than total mean). As a result, the cluster was considerable for high yield that its ecotypes may be well applied in a breeding project.

DISCUSSION

The results of ANOVA and cluster analysis represented considerable variation among the studied genotypes for the measured attributes. Regarding to widespread geographical parts of the milk thistle ecotypes habitats, the observed phenotypic variation may be influenced by effects of climatic conditions e.g. altitude from sea level

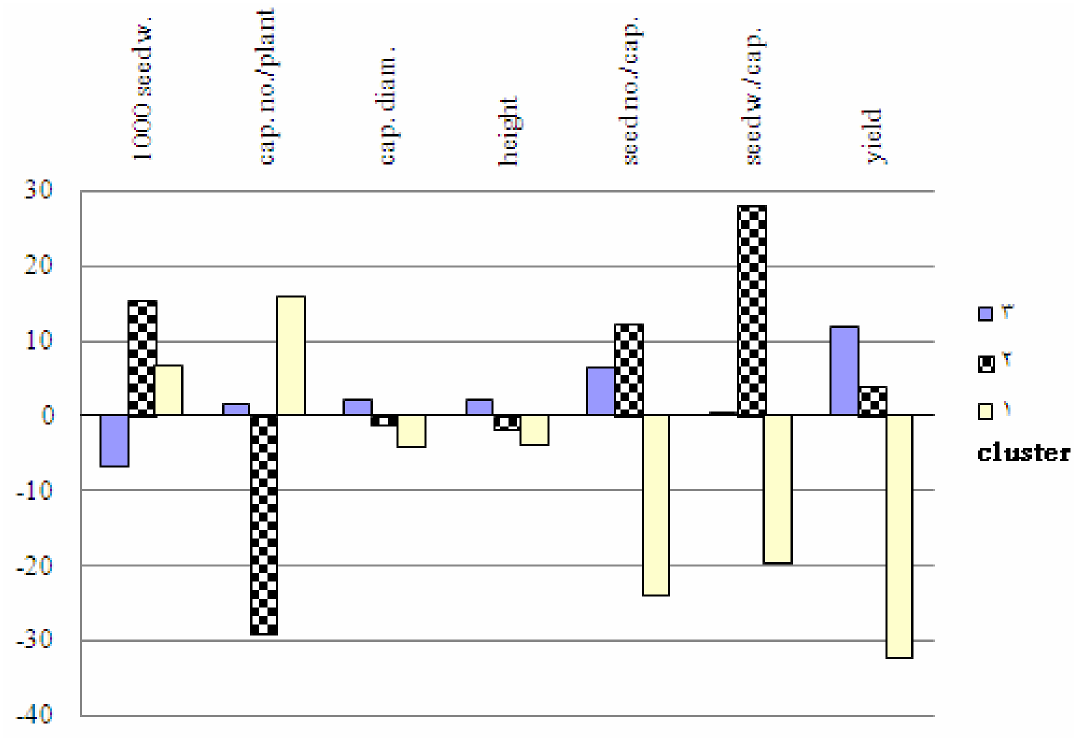

Figure 2. Values of differences of cluster means and total means for agoronomic traits.

and weather, and also different genotypic structure of the ecotypes. Ram et al. (2005) studied 15 milk thistle populations collected from six different countries, found large genotypic and phenotypic variation for morphological and pharmaceutical constituents. Gresta et al. (2006) assessed agronomic features in milk thistle at three various climate and suggested that the plant would be lesser affected by environmental factors and had a good adaptation to wide spectrum of environment conditions. It allows us therefore to adopt the same crop management techniques for different genotypes and environments. So it is possible, comparing and selecting high yield genotypes under same environmental conditions. According to suggestion of these researchers, it would be expected that north ecotypes e.g. Tatar, because of location of experimental site, have more yield than South ecotypes such as Behbahan. Of course, this conclusion is less considerately since it is essential to determine genotype-environment interaction by making multilocation experiments over several years.

Amounts of seed yield on the entries displayed that some ecotypes such as Tatar and Parsabad were superior in contrast to the introduced varieties, Budakalaszi and CNseeds. That suggests considerable potential for genetic resources to apply in milk thistle breeding projects. Also it emphasizes on the collection and conservation of all milk thistle genotypes through their habitats in Iran. Shokrpour et al. (2008) found significantly association among content of medicinal compounds and morphological attributes. They stated that ecotypes having more 1000 seed w. and lesser flowering date, capsule diameter and seed yield would have more silychristin and silybin and lesser silydianin. To put it more simply, the larger seeds had higher silybin and lower silydianin. As a result, identifying of superior genotypes for high quality and quantity of medicinal compounds may be feasible by selecting based on some associated traits such as yield and its components. This is so important in respect to difficulties of measuring medicinal compounds particularly be costly. Grouping the entries based on the studied attributes suggested that the ecotypes within each part of Iran had more similarity than the ecotypes from different parts. Hence it seems that the attributes would be partly influenced by climatic conditions. Shokrpour et al. (2007a) reported a good correspondence between habitats and grouping milk thistle genotypes based on morphological traits. However, classifying milk thistle ecotypes based on medicinal compounds had no correspondence to the geographical locations of the habitats (Shokrpour et al., 2007b).

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