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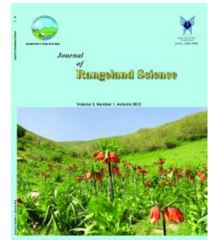

Full Length Article:

Evaluation of Seeds and Pods Variation of 5 Annual Medic *Medicago* Spp.

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Received on: 23/02/2013
Accepted on: 26/06/2013

Abstract. *Medicago* spp. is the most important forage crop in many regions of the world. Annual medics have distinct advantages over perennial *Medicago*. These species have faster growth rate in cultivation year, higher crude protein percentage, tolerance to pests and more vigorous seedlings. Morphological and physical aspects of pod and seeds of annual medics play a major role in the tolerance of the plants to over grazing and establishing in different type of rangelands i.e., plants with small seeds can establish better in heavy grazed rangelands, spiny pods establish better in dense compact clay soil, hard seeds increase seed bank of the soil and maintain the pasture for the next years. In order to study the variation in seeds and pods morphological traits of five annual *Medicago* spp. seeds of 50 accessions of *M. rigidula, M. polymorpha, M. orbicularis, M. radiata and M. minima*, provided from natural resources gene bank of Iran, were sown in pots. Data were collected for size of the seeds, spins and pods, number of seeds per pod, levels of hardseededness and elevation of the seed origin. Results showed that *M. rigidula* 1000-seed weight had the most variation. Largest seeds were 17 times bigger than the smallest one in this species. *M. polymorpha* showed the most variation in the elevation of seed origin (upper=2280 m, lower=5 m above sea level). *M. radiata* showed the most variation in the amount of hard seeds. *M. rigidula* showed the least amount of hard seeds (mean=59.93). *M. radiata* had the largest pods. *M. radiata* and *M. orbicularis* showed the highest variation in pod size. *M. polymorpha* showed the highest variation of spine length. The correlation between 1000-seed weight and the elevation of seed origin was positive and significant for *M. polymorpha* and negative and significant for *M. orbicularis*. Based on Ward clustering method, the five species were grouped into two clusters in a dendrogram. First cluster included *M. minima* and *M. polymorpha* and the second cluster included *M. rigidula, M. orbicularis,* and *M. radiata*. This result indicated that it may possible of interspecific hybridization between species of the same cluster.

Key words: Annual *Medicago*, Morphological aspects, Seed, Pod, Tolerance to grazing, Hardseededness.

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Introduction

Annual Medics are forage plants that play important role in dry land regions of the world (Walsh *et al.*, 2001). The *Medicago* genus, contains 83 species, is distributed mainly around the Mediterranean basin, and is a summer annual, bi-annual or perennial plant. This plant can be seen through the old world: all of Europe, a great part of Asia (including China, Korea and Taiwan, as well as the Indian sub-continent), North Africa, the islands of the Atlantic (the Canaries, Madeira) and throughout the United States. Latest taxonomic revisions of the genus *Medicago* identify 20 *Medicago* species from Iran (Mehregan, *et al.*, 2001 and Mehregan, *et al.*, 2002) among which *M. sativa* is perennial, *M. lupulina* is bi-annual and the others are annual.

Annual medics have distinct advantages over perennial medic. These plants have faster growth in the year of cultivation, higher crude protein content, tolerance to pests and more vigorous seedlings that make them attractive over perennial medic (Sanadgol and Malekpoor, 1993; Mirzaee Nadushan, 2002).

Most of the annual medic species show some degree of hardseededness and this makes them more valuable because hard seeds can stay alive in the soil for a long period of time, especially throughout drought periods, when the adverse condition is over they can grow and maintain a permanent pasture (Blumenthal and Ison, 1996; Cocks, 1992; Shabany, *et al.*, 2004). These plants produce large amount of seeds and in their morphological features are very variable (De-Haan and Barnes, 1998; Diwan, *et al.*, 1994). *Medicago* spp. grow in different climatic conditions in Iran from semi-alpine cold climatic condition to warm, similar to African, climate. These variations provide us a very valuable genetic diversity that researchers and breeders can use for breeding, improved varieties. Lack of the ability to absorb water in hard seeds in legumes is due to closure of hilum and other apertures of seed, hard seed coat and presence of some hydrophobic phenolic compounds (Heidary Shrif Abad and Tork Nejad, 2001).

The morphological aspects of pod and seeds of annual medic i.e. the size of the seeds, spins and pods and number of seeds per pod play an important role in the tolerance of the plants to over-grazing and range establishment in different type of rangelands. Small seeds and pods have higher survival rate in overgrazed pastures. It has been reported that smaller seeds survive better in digestive system of the livestock and larger pods have less seeds (Squella and Carter, 1993). There are evidences that species and ecotypes with spiny pods can establish better in clay soil with hard surface, which is common in Iran (Sanadgol and Carter, 1993).

The objective of this research is to study variations in morphological traits of seeds and pods of five annual medics, and to help breeders and researchers to introduce improved varieties in a shorter period of time.

Materials and Methods

Sampling method and measurement of traits

50 accessions for each one of 5 species of annual medic i.e. *M. polymorpha*, *M. rigidula*, *M. orbicularis*, *M. minima* and *M. radiata*, were collected from different part of the country.

The following traits were measured:

1. Thousand seeds weight: A sample of 4 x 100 clean seeds was weighed and the mean multiplied by 10.
2. Spine length: the average length of spine of largest coil of 30 pods of

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each accession were measured based on mm.

3. Diameter of pod: The average diameter of 30 pods of each accession were measured based on mm.
4. Seeds number per pod: Seeds number of 30 pods was noted down based on counting.
5. Hard seeds percent: The ratio of remaining not germinated seed to initial live seed on 100 x 2 surface-sterilized seeds sown in two Petri dishes and incubated in a 24°C germinator for two weeks.
6. Seed origin elevation: Seeds were collected from different elevations. The Latitude, longitude were also recorded as meter above sea level for each accessions.

Statistical analysis

Descriptive statistics including mean, Standard Error (SE), Standard Deviation (SD), minimum, maximum and Coefficient of Variation (CV), were calculated for each species. Data was analyzed using one way ANOVA. Means comparisons were made based on Duncan's Multiple Range Tests (DMRT). Seed size frequency distribution was plotted on 1000 seed weight for each species. Phenotypic correlations among elevation and other characteristics were estimated for all pair-wise combinations for each species. The standardized variables were used for cluster analysis (Ward method). The SAS9 software (SAS institute Inc.) was used for ANOVA and Duncan mean comparison test and Minitab 16 was used for cluster analysis.

Results

Descriptive statistics

Summary of descriptive statistics including mean, Standard Error (SE), Standard Deviation (SD), minimum, maximum and Coefficient of Variation (CV) for each species are presented in (Table 1). According to the results in (Table 1), *M. rigidula* and *M. minima* showed the highest and lowest variation for 1000-seed weight. *M. minima* with average values of 3.37 mm had the smallest pod and seed. For the elevation origin of the seed the *M. polymorpha* had higher range (5 to 2280 m above sea leval). *M. radiata* showed the most variation in the proportion of hard seeds (Max=100, Min=15.5, SD=30.96). *M. rigidula* and *M. radiata* with average values 59.93% and 15.31%, respectively, had the highest and lowest proportion of hard seeds. *M. radiata* and *M. orbicularis* showed the highest variation in pod size. *M. polymorpha* showed the highest variation in spine length (Min=1, Max=2.03, SD=0.42). *M. orbicularis* had the highest number of seed per pod and showed the highest variation for pod size (Min=11.30, Max=14.4, SD=1.12).

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Table 1. Summary of descriptive statistics including mean, Standard Error (SE), Standard Deviation (SD), minimum, maximum and Coefficient of variation (CV) for seed characteristics of each species

Trait	Species name	Mean	Min	Max	SE	SD	CV
Seed origin elevation	*M. minima*	434	5.0	1100	82.9	361.4	83.1
	M. orbicularis	969	5.0	1598	88.9	426.2	43.9
	M. polymorpha	927	5.0	2280	180.0	843.0	90.9
	M. radiata	1489	600	2270	88.6	386.4	25.9
	M. rigidula	1011	500	1670	92.4	392.2	38.7
1000-seed weight	*M. minima*	1.51	0.85	2.80	0.13	0.55	36.17
	M. orbicularis	3.69	2.25	4.50	0.12	0.56	15.15
	M. polymorpha	2.30	0.99	3.67	0.21	0.96	41.68
	M. radiata	4.26	2.11	6.20	0.21	0.90	21.10
	M. rigidula	3.99	1.98	6.00	0.32	1.36	34.03
Seed hardness	*M. minima*	68.98	16.00	90.70	5.40	23.52	34.10
	M. orbicularis	85.17	46.00	100.00	3.28	15.72	18.46
	M. polymorpha	70.45	24.00	100.00	4.64	21.77	30.90
	M. radiata	71.47	15.50	100.00	7.10	30.96	43.32
	M. rigidula	59.93	28.00	86.00	4.80	20.37	33.99
Pod size	*M. minima*	3.37	3.00	3.80	0.08	0.35	10.30
	M. orbicularis	12.62	11.30	14.40	0.23	1.12	8.89
	M. polymorpha	5.58	4.60	7.00	0.17	0.82	14.63
	M. radiata	15.31	13.30	17.50	0.27	1.19	7.80
	M. rigidula	5.88	5.20	6.52	0.09	0.38	6.39
Spine length	*M. minima*	1.44	1.05	1.98	0.06	0.26	17.89
	M. orbicularis	1.00	1.00	1.00	0.00	0.00	0.00
	M. polymorpha	1.40	1.00	2.03	0.09	0.42	30.35
	M. radiata	1.22	0.85	1.77	0.06	0.28	22.69
	M. rigidula	0.83	0.46	1.40	0.08	0.34	41.09
Seeds per pod	*M. minima*	4.23	3.40	5.20	0.14	0.59	13.92
	M. orbicularis	12.55	10.20	15.20	0.35	1.69	13.49
	M. polymorpha	3.56	3.00	4.20	0.09	0.42	11.72
	M. radiata	4.88	3.50	6.10	0.17	0.74	15.11
	M. rigidula	5.49	4.60	6.88	0.19	0.80	14.62

Seed size frequency

Results of seed size frequency distribution for 1000 seed weight are represented in (Figs. 1 to 5), for different species. Result showed that *M. rigidula* 1000-seed weight had the most variation (Fig. 1). Largest seeds in this species were 17 times bigger than that for the smallest one. *M. radiata* showed the least variation for this trait. Largest seeds in this species were 3.5 times bigger than that for the smallest one (Fig. 3). Diagram of frequency of 1000-seed weight for *M. minima* (Fig. 3), *M. rigidula* (Fig. 2) and *M. orbicularis* (Fig. 5) almost fit the normal distribution, but it did not fit normal distribution for *M. polymorpha* (Fig. 1) and *M. radiata* (Fig. 4).

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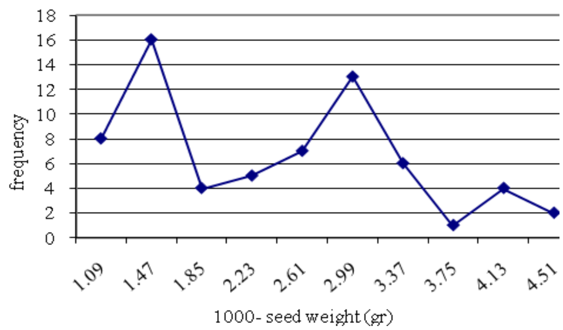

Fig. 1. Frequency of 1000- seed weight of M. *polymorpha*

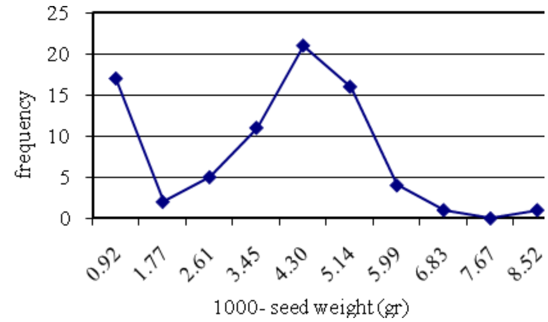

Fig. 2. Frequency of 1000- seed weight of M. *rigidula*

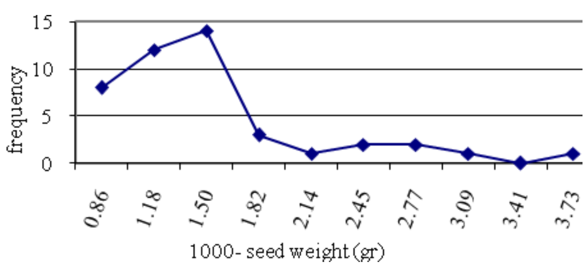

Fig. 3. Frequency of 1000- seed weight of M. *minima*

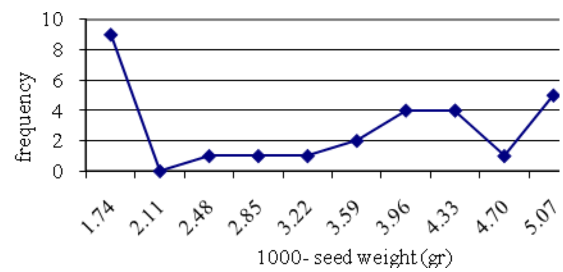

Fig. 4. Frequency of 1000- seed weight of M. *radiata*

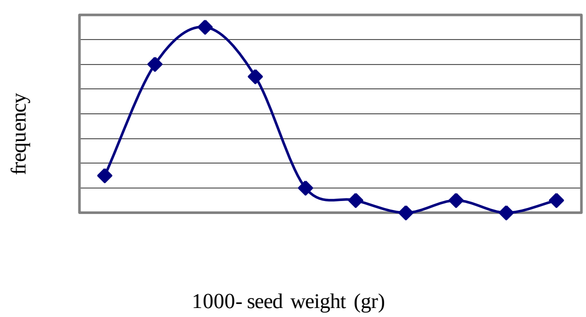

Fig. 5. Frequency of 1000- seed weight of M. *orbicularis*

Species means comparison

Mean comparisons of the traits (Table 2) showed that there was significant difference between elevations of seed origin. *M. orbicularis, M. polymorpha and M. rigidula* with elevations of 970, 925 and 1029 m above sea level, respectively, had no significant differences. But the seed origin elevation of *M. radiata* (1508 m) was significantly higher than *M. minima* with 447 m above sea level (Table 2).

For 1000-seed weight, *M. minima* and *M. polymorpha* with average values of 1.75 and 2.37 g, respectively, had significantly lower seed weight than the other 3 species. Spine length of *M. orbicularis* and *M.*

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rigidula with average values of 1 and 0.84 mm respectively, was shorter than the other species. All of the species had high hard seed proportion, with range 61.92 to 85.17 (Table 2). There were no significant differences between the proportions of hard seeds of the species.

Table 2. Means of seed origin elevation, 1000- seed weight, seed hardness, pod size, spine length and no. of seed per pod in different *Medicago* species, based on Duncan method

Species	Seed origin elevation (m)	1000-seed weight (g)	Seed hardness	Pod size (mm)	Spine length (mm)	No. of seed per pod
M. minima	447 c	1.57 b	70.42 a	3.33 d	1.40 a	4.20 cd
M. orbicularis	970 b	3.69 a	85.17 a	12.62 b	1.00 bc	12.55 a
M. polymorpha	925 bc	2.37 b	69.79 a	5.57 c	1.40 a	3.54 d
M. radiata	1508 a	4.28 a	73.32 a	15.26 a	1.20 ab	4.95 bc
M. rigidula	1029 b	3.83 a	61.92 a	5.93 c	0.84 c	5.57 b

The means with the same letter had no significant difference

Relationships between traits

The correlation coefficients between traits are presented in (Table 3). Results showed that the correlation between 1000-seed weight and the elevation of the seed origin was positively significant for *M. polymorpha* and mean of all species, suggested that the species grows in high elevation has heavy seeds .

M. rigidula was the only species that showed positive and significant correlation between hard seed proportion and seed origin elevation. Indicating that hard seed proportion is higher in higher elevations in this species.

For *M. rigidula* pod size and seed per pod had negative and significant correlation with elevation of the seed origin. Indicating that in lower places, *M. rigidula* had bigger pods with higher number of seeds per pods. There was no correlation between spine length and elevation in any of the 5 species. *M. polymorpha* and *M. radiata* spine length showed negative and significant correlation with pod size this was positive and significant in *M. minima*. The relationship between 1000-seed weight and pod size was positive and significant for all species. 1000-seed weight was negatively and positively correlated with seed hardness in M. *radiata* and *M. rigidula*, respectively. There was always a strong and positive relationship between seeds per pod and pod size.

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Table 3. Correlation between traits in different species

Trait	Species	Seed origin elevation	1000-seed weight(gr)	Seed hardness	Pod size	Spine length
1000- seed weight	*M. minima*	0.28				
	M. orbicularis	-0.45*				
	M. polymorpha	0.63**				
	M. radiata	-0.29				
	M. rigidula	-0.17				
	All species	0.46**				
Seed hardness	*M. minima*	-0.30	-0.19			
	M. orbicularis	0.10	0.02			
	M. polymorpha	-0.16	-0.40*			
	M. radiata	-0.29	0.61**			
	M. rigidula	0.45*	-0.67**			
	All species	-0.07	0.04			
Pod size	*M.minima*	-0.33	0.04	0.02		
	M.orbicularis	-0.08	0.06	-0.22		
	M.polymorpha	-0.18	0.01	0.39		
	M.radiata	0.14	0.02	-0.07		
	M.rigidula	-0.53**	-0.15	0.02		
	All species	0.43**	0.72**	0.18		
Spine length	*M. minima*	0.13	0.25	0.02	0.50*	
	M. orbicularis					
	M. polymorpha	0.12	0.07	-0.14	-0.48*	
	M. radiata	-0.27	0.13	-0.05	-0.46*	
	M. rigidula	-0.39	0.31	-0.82**	0.23	
	All species	-0.12	-0.30**	-0.16	-0.32**	
Seeds per pod	*M. minima*	0.09	0.27	-0.57*	-0.16	0.26
	M. orbicularis	0.00	0.07	-0.40*	0.87**	
	M. polymorpha	-0.27	-0.07	0.20	0.79**	-0.48*
	M. radiata	0.03	0.15	0.11	0.58**	0.10
	M. rigidula	-0.67**	-0.28	-0.29	0.58**	0.47*
	All species	0.03	0.35**	0.27*	0.53**	-0.40**

**,* significant difference at 1% and 5% respectively

Classification of species

Based on Ward clustering method, the five species grouped into two clusters in a dendrogram (Fig. 6). First cluster included *M. minima* and *M. polymorpha* and the second *cluster* included *M. rigidula*, *M. orbicularis* and *M. radiata*. This result indicates the possibility of interspecific hybridization between species of the same cluster.

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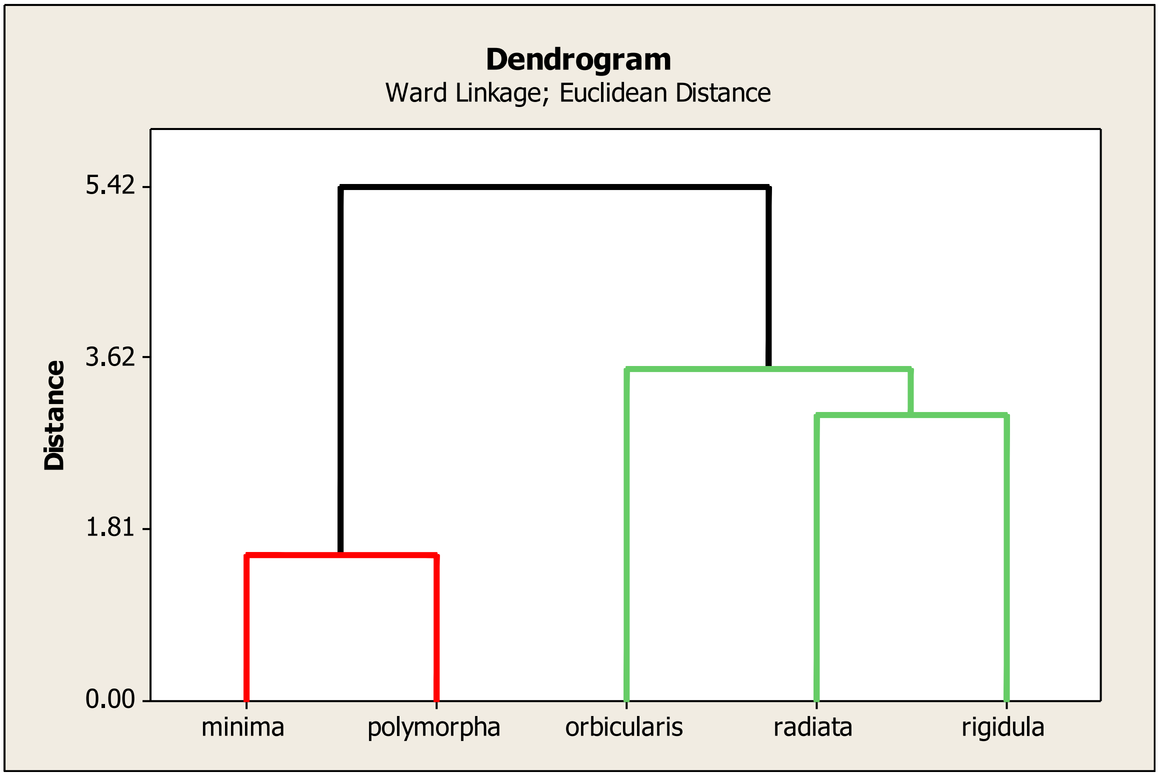

Fig. 6. Cluster analysis dendrogram based on different investigated traits

Discussion

Hardseededness is a trait that helps annul medics to withstand drought periods, this make them attractive over perennial species. Hard seeds increase soil seed bank and maintain a permanent pasture (Blumenthal and Ison, 1996; Cocks, 1992; Shabany, *et al.*, 2004). This is a valuable characteristic, especially in the pastures with irregular rainfall pattern. But when there is ample rainfall, for higher number of seedlings and synchronize seedling emergence, lower hard seed proportion is more suitable. Considering the climatic condition of the pasture, high or low hard seed proportion is needed. According to the results there is no significant difference between hard seed proportion of different species (Table 2) but there are suitable variation in hard seed proportion of each species (Table 1) and it is possible to choose accessions with different hard seed proportions. Some published reported showed that hardseededness is more related to environmental condition and less to genetic component of the plant. Mirzaee Nadushan (2002) reported that impermeable layers of seed coat produces at the end of the growing season, if due to environmental condition, seed growth and maturity be stopped, there would be less hard seed production. This researcher stated that those environmental conditions under which seeds can mature in a longer period of time, causes more hard seed production. Shamsi (2012) performing a study on the effect of different fertility treatments, foliage harvesting and irrigation on seed-hardness, concluded that number of hard seeds in *M. scutellata* was lower in none-foliage harvesting. There is some reports that hard seed characteristic in legumes might be a kind of beneficial biological adaptation which developed during the long-term co evolution with wild animals; it is a kind of special defense

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mechanism and had important ecological significance (anonymous, 2012). In order to choose those populations that their hard seed proportion have a genetic basis, it can be suggested that those seed accessions that collected in the same environmental conditions and show different hard seed proportions are more reliable. Other researchers reported the same results as our results about variation of hard seed proportion in annual *Medicago* species in different research studies (Taylor, 1993; Taylor, 1996; Lloyd *et al*., 1997).

Variation for 1000-seed weight specifies small and large seeded ecotypes. Depending on pasture type, environmental condition and grazing policy, ecotypes with different seed size is needed. Populations with small seeds and pods are suitable for heavy grazed pastures (Mirzaee Nadushan, 2002, Cocks and El Moneim, 1987; Squella and Carter, 1993). Mouissie *et al*. (2005) reported that there is a negative correlation between seed's size and weight and seed survival after passing through digestive system of animal. Squella and Carter (1993) reported that smaller seeds survive better in digestive system of livestock and larger pods provide less number of seed. These researchers also reported that survival rate of large seeds after passing through digestive system of the livestock was 5% and this was 60% for small seeds. Sanadgol and Malekpoor (1993) reported that those seeds with less than 4 mg weight, can survive heavy grazing more than heavier seeds. On the other hand large seeds usually produce more vigorous seedlings (Carleton and Cooper, 1972). Ariapour and Tork nejad (2011) performing a study on effect of seed size on yield of 6 annual *Medicago* spp. concluded that dry matter production, number of internodes and plant height was significantly higher in treatments with large seeds.

Results showed that there was negative and significant correlation between *M. rigidula* and *M. polymorpha* for1000-seed weight of this means that smaller seeds have higher proportion of hard seed. This correlation was positive in *M. radiata* so ecotypes with smaller seeds show lower proportion of hard seed in this species. These information can guide, those who are involve in forage production, to choose the suitable ecotypes regarding the condition they are dealing with. There was a positive significant correlation between size of the pod and number of seed per pod in all of the species except *M. minima*. This result is not in agreement with the report of Squella and Carter (1993) which concluded that larger pods contain less seeds.

M. polymorpha had the longer spine and showed the most variation in spine length (Max=2.03, SD=0.09) this provide us short and long spine accessions (Table 1). There are evidences that species and ecotypes with spiny pods can establish better in clay soil with hard surface (Sanadgol and Malekpoor, 1993; Squella and Carter, 1993). Considering that this kind of soil is very common in our pastures in Iran, ecotypes with longer spines can be used in these areas. According to the results of statistical parameters (Table 1) *M. polymorpha* showed the most variation in seed origin elevation (Max=2280, Min=5.0, SD=843). This shows the ability of adoption of this species to different type of environmental conditions. This is in agreement with numerous studies including Young *et al.* (1996) which reported that *M. polymorpha* had a very diverse gene pool and Sanadgol and Malekpoor (1993) and Heyn (1984) which reported that the variation of environmental conditions that this species can grow is higher than any other *Medicago* species. But it seems that seed collection of *M. polymorpha* in natural resources gene bank of Iran does not cover all of the genetic diversity of the

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species, because diagram of 1000-seed weight frequency does not fit normal distribution in this species (Fig. 1). Considering high genetic diversity of the species it is highly recommended that more collecting should be done all over the country.

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ارزیابی تنوع موجود در بذر و میوه ۵ گونه یونجه یکساله

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چکیده

یونجه (***Medicago***) یک از قدیمی‌ترین و مهمترین گیاهان مرتعی بوده و در بسیاری از کشورها مهمترین گیاه علوفه‌ای محسوب می‌شود. یونجه‌های یکساله از جهات مختلفی بر یونجه‌های چندساله برتری دارند. ویژگیهای ظاهری بذر مثل اندازه بذر، خار و غلاف، با مقاومت در برابر چرا و امکان استقرار این گیاه در مناطق آب و هوایی مختلف رابطه دارد. برای بررسی تنوع موجود در ویژگیهای مورفولوژیکی پنج گونه یونجه،۵۰ نمونه بذری از گونه‌های ***M. orbicularis***, ***M. polymorpha***, ***M. rigidula***, ***M. radiate*** و ***M. minima*** موجود در بانک ژن منابع طبیعی موسسه تحقیقات جنگلها و مراتع کشور، در گلدان کشت شده و ویژگیهای ظاهری بذر و غلافها ثبت شد. نتایج نشان داد که وزن هزار دانه ***M. rigidula*** بیشترین تنوع را داشته است. ***M. polymorpha*** بیشترین تنوع را در ارتفاع محل جمع‌آوری داشت. گونه ***M. radiate*** بیشترین تنوع را در درصد سختی بذر نشان داد و همچنین بزرگترین اندازه غلاف را داشت. همبستگی بین وزن هزار دانه و ارتفاع محل جمع‌آوری در ***M. polymorpha*** مثبت و معنی‌دار و در ***M. orbicularis*** منفی و معنی دار بود. بر اساس روش Ward گونه‌ها در دو کلاستر گروه‌بندی شدند کلاستر اول شامل ***M. minima*** و ***M. polymorpha*** و کلاستر دوم شامل ***M. rigidula***, ***M. orbicularis*** و ***M. radiate*** بود. این نتایج نشان می‌دهد که امکان هیبریداسیون بین گونه‌ای در گونه‌های قرار گرفته در هر کلاستر وجود دارد.

کلمات کلیدی: یونجه یکساله، مشخصات مورفولوژیکی، بذر، غلاف، سختی بذر، مقاومت به چرا