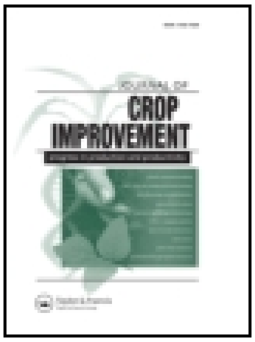

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Cold-tolerant germplasm identified in annual medics (*Medicago* spp.) collection of National Plant Gene Bank of Iran

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ABSTRACT

Annual medics are feed legumes that can be used as a cover crop or green manure. Species such as *Medicago scutellata* are sensitive to cold stress. To identify cold-tolerant germplasm, 351 accessions of Iranian annual medics, belonging to 13 species, were evaluated. Growth rate was scored for each accession from autumn to spring. Based on first-year results, *M. coronata*, *M. laciniata*, *M. littoralis*, *M. turbinata*, and *M. scutellata* were identified as cold-sensitive species, whereas four species [*M. minima* (5 accessions), *M. noeana* (2 accessions), *M. rigiduloides* (4 accessions), and *M. rigidula* (5 accessions)] were found to be cold-tolerant and tested further using a randomized complete-block design with three replications. Significant differences ($p < 0.01$) among accessions for agronomic traits (forage-, seed-, and pod-yield; percentage of green cover) were detected. *M. rigiduloides*, *M. rigidula*, *M. minima*, and *M. noeana* were cold-tolerant in descending order. Such cold-tolerant accessions can be used in breeding programs to develop cold-tolerant varieties.

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Abiotic stress; Medicago; forage; germplasm evaluation; multivariate analysis

Introduction

Annual *Medicago* species (annual medics) are forage legumes that can be used in ley-farming systems (the growing of grass or legumes in rotation with grain crops as a soil conservation measure) as well as in sustainable agriculture systems. These plants originated in western Asia and the Mediterranean region. They are used for feed production, as pasture or for soil improvement, and are widely distributed throughout the world (Crawford, Lake, and Boyce 1989; Lesins and Lesins 2012; Leilah and Al-Khateeb 2005). In general, medics require a Mediterranean climate with 250–500 mm of mainly winter rain.

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Therefore, these legumes have been used most successfully in cereal-legume crop rotation systems, such as those in Australia (53 million ha) (Angus and Peoples 2013), where winter rainfall ranges from 250 to 500 mm (James and McCulloch 1990; Romesburg 2004). Annual medics can be used under irrigated conditions in addition to drylands (rain-fed farming systems) (Entz et al. 2002; Angus and Peoples 2013).

The minimum temperature for annual medics growth is 3–7°C and the maximum is 30–35°C. Soil and air temperatures, respectively, in the range of 6–10°C and 15–30°C, are suitable for seed germination (Dehaghi and Sanavy 2003). Therefore, annual medics grow best in cool, autumn temperatures.

Krall, Groose, and Sobels (1996) showed that *M. rigidula* had higher potential for winter survival compared with *M. polymorpha* L. and *M. truncatula* Gaertn. Other researchers found *M. rigidula* also to be more cold-tolerant than other annual medics (Abd El-Moneim and Cocks 1986; Cocks and Ehrman 1987). Cocks and Ehrman (1987) reported that in two severe winters (1983 and 1984), *M. rigidula* exhibited more cold tolerance than other annual medics. Furthermore, Walsh et al. (2001) showed that *M. rigidula*, compared with 17 lines and cultivars of annual medics, consistently produced the highest level of dry matter and had the highest potential for further development in the dryland cropping system in southeastern Wyoming. Hekneby, Antolín, and Sánchez-Díaz (2006) showed that under laboratory conditions, *M. polymorpha* was more cold-tolerant and had higher forage production compared with four annual legumes, including *M. truncutula*, which is native to the Mediterranean region.

Some Iranian annual medics have been evaluated for cold tolerance. Dehaghi and Sanavy (2003) indicated that *M. polymorpha* and *M. rigidula* might be better suited for ley-farming systems in cold and temperate zones than *M. radiate* L. Tork Nejad (1998) showed that *M. radiata* and *M. rigidula* could tolerate as low as −10°C in western Iran.

There exists one report that deals with cold tolerance of Iranian medics under dryland conditions (Alizadeh and Sadeghzadeh 2010), but there is no report that deals with cold tolerance under irrigated conditions. Because of its high feed production, *M. scutellata* Mill is cultivated mainly in rotation with cereals in the southwestern region of Iran that has a temperate climate. In some years, this species cannot tolerate winter cold stress. Hence, the objective of the study was to identify, under irrigated conditions, cold-tolerant germplasm of annual medics maintained in Iran's National Plant Gene Bank, which can serve as an alternative to *M. scutellata*. The cold-tolerant materials identified in this research could be used in annual medics breeding programs.

Table 1. Species, number of accessions of each, and accession origin of the evaluated materials in the study.

Species	Number of accessions	Province of the accession origin
Medicago constricta	3	Azarbaijan sharghi, Kermanshah,
M. coronata	1	Kermanshah, Fars, Lorestan,
M. lacinata	4	Boshehr, Fars,
M. littoralis	4	Kermanshah, Boshehr Mazandaran,
M. minima	67	Azarbaijan sharghi, Kermanshah, Fars, Gilan, Hamedan. Lorestan, Khozestan, Mazandaran, Semnan
M. noeana	19	Azarbaijan gharbi, Kermanshah, Fars, Hamedan, Lorestan
M. orbicularis	47	Azarbaijan gharbi, Azarbaijan sharghi, Kermanshah, Boshehr, Fars, Ilam, Khozestan, Lorestan, Mazandaran
M. polymorpha	4	Azarbaijan gharbi, Kermanshah, Fars, Hamedan, Ilam, Kerman, Lorestan, Mazandaran, Tehran
M. radiata	36	Azarbaijan gharbi, Kermanshah, Fars, Hamedan, Kordestan, Lorestan
M. rigidula	77	Azarbaijan gharbi, Azarbaijan sharghi, Kermanshah, Fars, Hamedan, Khorasan, Kordestan, Lorestan, Mazandaran, Semnan
M. rigiduloides	38	Azarbaijan gharbi, Azarbaijan sharghi, Kermanshah, Fars, Hamedan, Kerman, Lorestan, Mazandaran, Zanjan
M. scutellata	5	Boshehr, Fars, Khozestan, Lorestan
M. turbonita	1	Kermanshah
Total	351	

Materials and methods

Three-hundred and fifty-one accessions of annual medics, belonging to 13 species, held by the National Plant Gene Bank of Iran (Table 1), were planted by hand in single-row plots, 2 m long and 0.5 m apart, near Zanjan (36°31′ N, 48°47′ E, 1770 m), Iran, in September 2006. These materials originated from regions with cold climate or from high-elevation regions of Iran and had good vegetative growth (Abbasi 2015; Abbasi et al. 2014). As *M. scutellata* is cultivated in Lorestan province (western Iran), Lorestan landrace was used as control in the first year of testing. All seeds were scarified with a paper file to remove seed hardness. Plots were arranged in 39 rows in each of 10 blocks. The Lorestan landrace of *M. scutellata* was planted in every 10th row as a check. The field was irrigated every 8 days from September to October and in spring from March to June. During the autumn and winter, the growth rate was evaluated visually by comparing the relative amount of growth of each accession to that of the nearest check using a 1–5 scale (2.5 = growth rating for the check), where 1–2 = growth < the nearest check, and 3–5 = growth >the nearest check (Caddel 2011). Plants in none of the check plots survived after winter; hence, to evaluate the growth rate in the spring among surviving accessions, the score was given from 1 (for the least growth) to 5 (for the highest growth). Sixteen accessions belonging to four species (five *M. minima*, two *M. noeana*, four *M. rigiduloides*, and five *M. rigidula*) were selected on the basis of the first-year results, which were evaluated in the following year. The 16 accessions were planted in a randomized complete-block design with three replications. The plots consisted of four 2-m-long rows, 0.5 m apart. The date of planting for both years was 15 September. In the second year, forage yield was recorded from the first two rows, whereas seed and pod yields were recorded from the other two rows. Pods were manually harvested from each plot and dried under natural airflow. They were threshed in a hammer mill and seeds were cleaned using sieves and air. Weights of dried seeds and pods were recorded for each plot. Other agro-morphological traits were measured using International Plant Genetic Resources Institute (IPGRI 1991) descriptors.

Data analysis

Analysis of variance and Duncan's Multiple Range Test for agronomic traits (growth rate after snow, percentage of green cover in plot, forage yield, pod yield, seed yield, and seed/pod ratio) of the selected annual medics (16 accessions) were conducted. Data were normalized by subjecting them to square root transformation for variance analysis. The mean of each trait per accession (from three replications) was adopted as a datum in the cluster analysis based on between-groups linkage method. Extraction method employed in factor analysis was principal component analysis and rotation method was Varimax with Kaiser Normalization. Pearson's correlation analysis was used to determine relationships between agronomic traits and altitudes of the collection sites for the accessions. These analyses were performed via SPSS version 15 (Leilah and Al-Khateeb 2005).

Results

Figure 1 represents metrological data for the experimental field in the first and second years. A mean of 136.5 as number of freezing days for the two years with an absolute minimum temperature of −29.4 implies severe cold conditions existed in the experimental field (Figure 1).

In the first year, all species had vegetative growth in the fall before the beginning of snow and cold conditions (Table 2). The mean growth scores for each species showed that *M. rigiduloides*, *M. rigidula*, *M. minima*, and *M. turbinate* had growth score of 2.5 or higher; the mean score for *M. scutellata* accessions, non-check plots (51TN01166, 51TN01355, 51TN02006, 51TN02098, and 51TN02100), was 1.6 (Table 2). *M. rigiduloides* and *M. rigidula* accessions showed a growth score of 3.3 or higher during the fall season. *M. constricta* and *M. littoralis* had a mean growth score of one, the lowest among all species (Table 2).

In the first winter, *M. coronata*, *M. laciniata*, *M. littoralis*, *M. turbinata*, and *M. scutellata* (both checks and experimental plots) could not tolerate the cold stress and all the accessions of these species died because of severe frost (Table 2). The surviving accessions in *M. rigiduloieds*, *M. rigidula*, *M.*

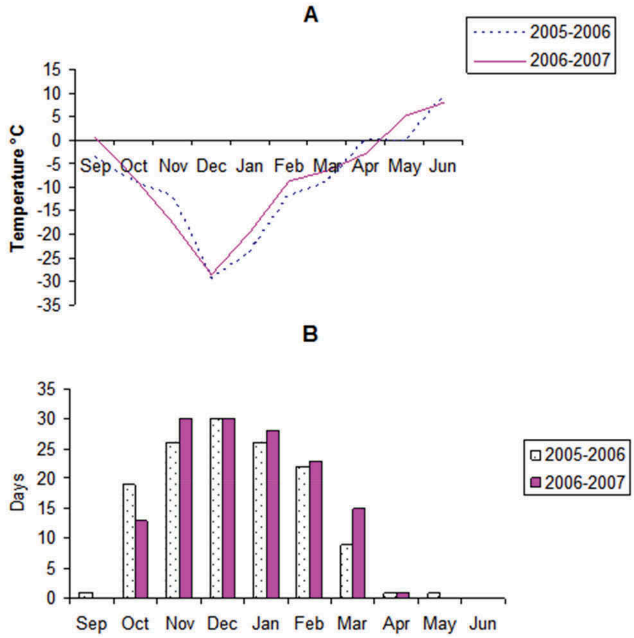

Figure 1. Distribution of absolute minimum temperature (a) and numbers of freezing days (b) during 2005–2006 and 2006–2007 cropping seasons, in the experimental field.

Table 2. Mean of growth rate score before and after snow (in fall and following spring) and remained accessions in each species in the first year.

Species	Growth rate before snow†	Growth rate after snow‡	Percentage of surviving accessions in the spring
Medicago constricta	1.0	1.0	33.3
M. coronata	1.6	0	0
M. laciniata	1.4	0	0
M. littoralis	1.0	0	0
M. minima	2.7	2.5	34.9
M. noeana	2.4	3.1	36.8
M. orbicularis	1.5	2.0	2.6
M. polymorpha	1.6	3.5	5.3
M. radiata	2.1	3.0	6.9
M. rigidula	3.3	3.1	48.6
M. rigiduloides	4.0	3.2	77.8
M. scutellata	1.6	0	0
M. turbinata	2.5	0	0

†On a 1–5 score, where 1–2 = growth less than the nearest check(s) and 3–5 = growth more than the nearest check(s); 2.5 = check (control) growth.

‡On a 1–5 score, where 1 = the least growth, 2 = low growth, 3 = moderate growth, 4 = high growth, and 5 = the highest growth (visual basis).

noeana, and *M. minima* were 77.8%, 48.6%, 36.8%, and 34.2%, respectively (Table 2). Although percent survival for *M. constricta* was 33.3%, it had only three accessions, which cannot be considered a representative of the entire species.

M. rigiduloides, *M. rigidula*, *M. minima*, *M. noeana*, *M. polymorpha*, and *M. orbicularis* showed high growth rate in the first spring. Fourteen accessions had the highest growth rate (score 5) and 18 accessions had a score of four for growth rate in the first spring. Six accessions of *M. rigidula* and five accessions of *M. rigiduloides* had a growth rate score of 5 (data not shown). The mean growth rate varied from 1 in *M. constricta* to 3.5 in *M. polymorpha* (Table 2). Although, in *M. polymorpha*, the mean score was 3.5, it was based on only two accessions. In *M. rigidula* (36 accessions) and *M. rigiduloides* (28 accessions), the mean growth rates were 3.1 and 3.2, respectively (Table 2). The mean score was 2.5 for *M. minima* and 3.2 for *M. noeana*. The least score was recorded for *M. constricta*, with a mean of 1 (Table 2). A total of 99 out of 351 accessions of eight species survived in the field and continued to grow in the first spring.

In the second year, all accessions in the field showed a good vegetative growth during the fall and before onset of snow. Significant genotypic differences ($p < 0.01$) existed for all the evaluated traits, except seed/pod ratio (Table 3). The 16 accessions were grouped for each of the traits by means of Duncan's Multiple Range Test. *M. rigidula* (51TN00787 and 51TN00933) and *M. minima* (51TN01844) were located in group *e* (the highest level) for growth rate, whereas 51TN01914 (*M. rigidula*) and 51TN00656 (*M. minima*) appeared in group *a*, with the lowest value for the trait (Table 4).

For the percentage of green cover in the plot, 51TN01851 of *M. rigidula*, and 51TN01853 belonging to *M. rigiduloides* were located in the highest value group (group *f*). Accession 51TN02004 appeared in group *a* (the lowest level).

For biological yield, seven accessions were categorized in group *a* (the lowest level). Accessions 51TN01842 and 51TN01853 of *M. rigiduloides* were located in group *d* (the highest group, with mean of 505.7 and 568.7 g/plot, respectively). For pod yield, the germplasm was categorized into six groups, where 51TN01853 was located in the 6th and the highest group (*d*), with a mean of 405.5 g/plot). The germplasm for seed yield appeared in 11 groups and accession 51TN01853 was located in the highest group (*g*), with a mean of 89.6 g/plot.

Clustering of accessions

The germplasm was grouped into four clusters (Figure 2). The first cluster consisted of seven accessions: two of *M. noeana* (51TN01959 and 51TN01992), four of *M. minima* (51TN00656, 51TN01022, 51TN01397, and 51TN02004) and one of *M. rigidula* (51TN01914) (Figure 2). The second cluster consisted of three accessions: one each of *M. rigiduloides* (51TN00788), *M. rigidula* (51TN02047), and *M. minima* (51TN00933) (Figure 2). The third cluster contained three accessions of *M. rigidula* (51TN00787, 51TN01844, and 51TN01851) and two accessions of *M.*

Table 3. Results of analysis of variance (*F* test) for agronomic traits of selected annual medics in the second year.

Traits	Source of variation	Degrees of freedom	Mean square
Growth rate (score)	Replications	2	27.8**
	Treatments	15	11.94**
	Error	30	3.93
Percentage of green cover (%)	Replications	2	2140.3**
	Treatments	15	1783.04**
	Error	30	353.96
Forage yield (g/m^2)	Replications	2	7.4 ^{ns}
	Treatments	15	184.5**
	Error	30	22.1
Pod yield (g/m^2)	Replications	2	2.0 ^{ns}
	Treatments	15	108.9**
	Error	30	12.1
Seed yield (g/m^2)	Replications	2	0.33 ^{ns}
	Treatments	15	24.74**
	Error	30	3.3
Seed pod ratio (g/m^2)	Replications	2	0.00^{ns}
	Treatments	15	0.07 ^{ns}
	Error	30	0.05

**Significant at the 1% probability level; ns: not significant.

rgiduloides (51TN01048 and 51TN01842) (Figure 2). The fourth cluster included a single accession of *M. rigiduloides* (51TN01853).

The results of factor analysis showed that 84.9% of diversity in the accessions was explained by the first two factors. The forage yield and the seed yield had the highest effect on factor one, whereas factor two was mainly affected by seed/pod ratio (Table 5). The distribution of 16 accessions is shown in a biplot resulting from factors one and two in the factor analysis (Figure 3).

Discussion

Although *M. scutellata* reportedly showed good vegetative growth in the temperate regions in a different study (Dori 2008), the mean growth rate of this species in the current study (Table 2) was adversely affected in the Zanjan region under cold climate (Figure 1). Similarly, most of the accessions belonging to *M. orbicularis*, *M. polymorpha*, and *M. radiate* could not tolerate the cold stress in the first year (Table 2). On the contrary, *M. rigidula*, *M. noeana*, and *M. minima* showed a strong ability to survive after snow (Table 2). The growth rate score was higher in these four species compared with others in the first spring. None of the 33 controls and 5 non-control accessions of *M. scutellata* could tolerate the cold conditions during the first winter. Therefore, no accession from these 38 plots of *M. scutellata* was selected for planting in the second year. Our results are in line with the findings of other investigators (Krall, Groose, and Sobels 1996; Abd El-Moneim and Cocks 1986; Cocks and Ehrman 1987) in that *M. rigidula* was

Table 4. Means of agronomic traits for the 16 selected accessions of annual medics and location altitude at which each accession was collected.

Accession number	Species	Altitude of collection site (m)	Growth rate	Percentage of green cover	Forage yield (g/plot)	Pod yield (g/plot)	Seed yield (g/plot)	Seed pod ratio
51TN00656	*M. minima*	†	$4^{a‡}$	23.3^{abcd}	27.8^{a}	14.4^{a}	5.7^{ab}	0.27^{ab}
51TN00787	*M. rigidula*	1450	9^{e}	66.7^{ef}	343.3^{bcd}	135^{abc}	40.9^{bcdef}	0.32^{bc}
51TN00788	*M. rigiduloides*	1500	5.7^{abcde}	23^{abcd}	128.3^{ab}	83.8^{ab}	21.7^{abcde}	0.26^{ab}
51TN00933	*M. minima*	1600	9^{e}	45^{abcdef}	232.7^{abc}	22.7^{a}	19.6^{abcd}	0.55^{c}
51TN01022	*M. minima*	1330	6^{abcde}	40.3^{abcdef}	90.7^{a}	29.8^{a}	8.72^{ab}	0.30^{abc}
51TN01048	*M. rigiduloides*	1220	8.3^{cde}	60^{ef}	427^{cd}	250.3^{c}	56^{f}	0.23^{ab}
51TN01397	*M. minima*	1200	5^{abcd}	8.7^{ab}	38.6^{a}	15.2^{a}	3.6^{a}	0.14^{ab}
51TN01842	*M. rigiduloides*	1740	8.7^{de}	58.3^{def}	505.7^{d}	237.7^{c}	55^{ef}	0.24^{ab}
51TN01844	*M. rigidula*	1890	9^{e}	51.7^{bcdef}	338.7^{bcd}	204.7^{bc}	53.5^{def}	0.26^{ab}
51TN01851	*M. rigidula*	2040	8^{bcde}	73.3^{f}	400^{cd}	212^{bc}	47.8^{cdef}	0.22^{ab}
51TN01853	*M. rigiduloides*	1800	8.7^{de}	75^{f}	568.7^{d}	405.5^{d}	89.6^{g}	0.22^{ab}
51TN01914	*M. rigidula*	960	4^{a}	11.3^{ab}	42.6^{a}	56.9^{a}	9.8^{ab}	0.11^{ab}
51TN01959	*M. noeana*	1700	8.3^{cde}	20^{abc}	9.6^{a}	7.7^{a}	1.7^{a}	0.14^{ab}
51TN01992	*M. noeana*	1560	4.3^{ab}	8.7^{ab}	5^{a}	0^{a}	0a	0^{a}
51TN02004	*M. minima*	1050	7.7^{abcde}	4.6^{a}	15.3^{a}	5.38^{a}	0.63^{a}	0.15^{ab}
51TN02047	*M. rigidula*	1000	4.7^{abc}	32.3^{abcde}	167.1^{ab}	78.4^{ab}	18.1^{abc}	0.22^{ab}

†No data for this accession.

‡Means, within each column, followed by the same letter(s) were not significantly different at 5% probability level.

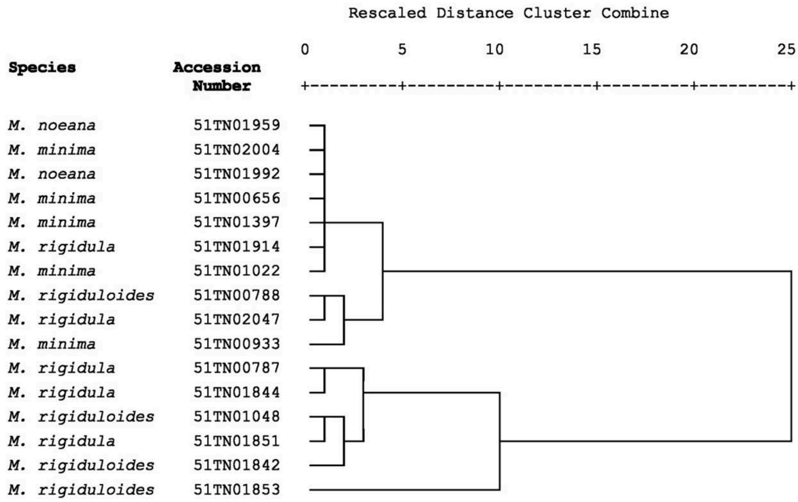

Figure 2. Produced dendrogram based on average linkage (between groups) method using of agronomic traits in annual medics accessions in the second year.

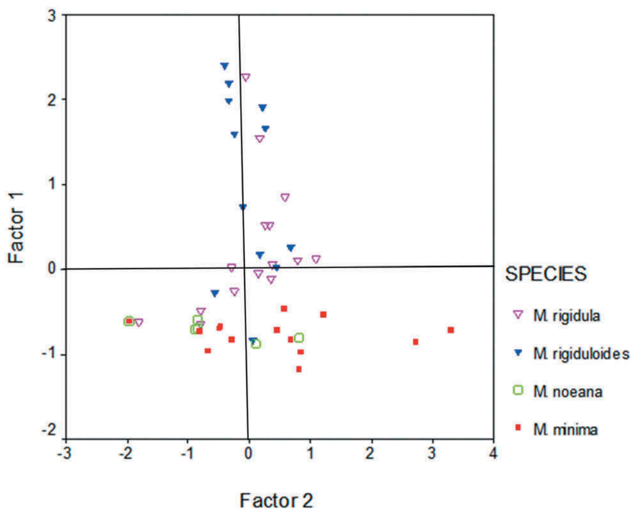

Figure 3. Distribution of annual medic accessions in the produced bi-plot based on two first factors of factor analysis in the second year.

more cold-tolerant than other annual medics. In addition, our results confirmed the findings of Dehaghi and Sanavy (2003) and Tork Nejad (1998) concerning high potentiality of *M. rigidula* for use in breeding programs in cold climates. Furthermore, the present study identified *M. rigiduloieds* as cold-tolerant, in addition to the abovementioned species. This research constitutes the first report of cold tolerance in the Iranian collection of annual medics under irrigated conditions.

Table 5. Results of factor analysis on agronomic traits in the selected annual medics (second-year materials).

Variables	1	2
	Factor	
Total eigenvalues	3/3	1/8
Percent of variance	55/7	29/2
The cumulative percent of variance	55/7	85/0
	Eigenvectors	
Growth rate	0/38	0/69
Percentage of green cover	0/69	0/57
Forage yield	0/91	0/32
Pod yield	0/98	0/05
Seed yield	0/96	0/19
Seed/pod ratio	0/01	0/90

Sixteen selected accessions tested in the second year, which belonged to *M. rigidula*, *M. rigiduloides*, *M. minima*, and *M. noeana*, originated in areas that were between 960 m above sea level (masl) in north (Mazandaran) and 2040 masl in the northwest (Hamedan) of the country (Table 4, and Abbasi 2015). As shown in Table 4, accessions 51TN01844, 51TN01851, and 51TN00787 belonging to *M. rigidula*; 51TN01853 and 51TN01842 belonging to *M. rigiduloides*; and 51TN00933 belonging to *M. minima* showed high values for most of the traits in Duncan's Multiple Range Test. Therefore, these accessions can be considered cold-tolerant materials. Although *M. noeana* accessions originated in the upland regions with cold climate in the northwest of the country (Abbasi et al. 2014), they did not show high values for the evaluated traits; in other words, accessions of *M. noeana* had low production potential (Table 4).

The first-cluster accessions represent mildly cold-tolerant materials (Figure 2). These accessions had the lowest values for most of the evaluated traits (Table 4). Accession 51TN01914 of *M. rigidula*, which appeared in this cluster, had been collected from the northern region of Iran in Mazandaran province that has temperate climate (Abbasi et al. 2014). The accessions in the second cluster (51TN00788 and 51TN00933) were those that showed the highest score for growth rate. The germplasm included in this cluster can be considered cold-tolerant. The third and the fourth clusters represented strongly cold-tolerant accessions (Figure 2). In the third cluster, the accessions belonging to *M. rigidula* (51TN00787, 51TN01844, and 51TN01851) were collected from upland (1450–2040 masl) provinces of Hamedan and Kermanshah (western Iran) with cold climate (Table 4), and *M. rgiduloides* accessions (51TN01048 and 51TN01842) were collected from the northwestern region of the country (Abbasi et al. 2014). The accessions of the third cluster possessed the highest values for most of the evaluated traits. For example, for growth rate, accessions 51TN00787 and 51TN001844 located in *e* group and for other traits, accessions 51TN01048, 51TN01842, and 51TN01851 showed the highest values (Table 4). The accession in the fourth cluster (50TN01853) originated from Hamedan province (1800 masl) (Abbasi et al. 2014) (Table 4). Except for growth rate and seed/pod ratio, this accession showed the highest values for all other traits (Table 4). The materials of the third and fourth clusters, which belong to *M. rigidula* and *M. rigiduloides*, can be considered highly cold-tolerant germplasms and can be deployed in breeding programs. Alizadeh and Sadeghzadeh (2010) reported that accession 51TN01851 belonging to *M. rigidula* was the most cold-tolerant genotype among Iranian medics under dryland conditions. In addition to accession 51TN01851, our results identified some highly cold-tolerant accessions belonging to *M. rigiduloides* (51TN01853, 51TN01842, 51TN01048) too.

Based on the results of factor analysis (Table 5), the first factor was mainly affected by traits such as percentage of green cover, forage yield, pod yield, and seed yield; those accessions with high value for these traits are located in the top section of the biplot (Figure 3) and are considered highly cold-tolerant germplasms. They belong to *M. rigidula* and *M. rigiduloides* (Figure 3). Some accessions of *M. minima* are located in the lowest part of the biplot and the accessions belonging to *M. noeana* appeared a little bit above them (both species were identified as mildly cold-tolerant) (Figure 3). As Table 5 shows the factor 2 was mainlyaffected by seed/pod ratio trait. Therefore, those accessions whichappeared on high values of factor 2 (Figure 3) of the biplot had highvalue for seed/pod ratio trait. Confirmed the result of cluster analysis and provided more information on the accessions than cluster analysis.

Correlation analysis showed a significant positive relationship between geographical altitude of collecting site and growth rate (r = 0.598, $p < 0.05$), percentage of green cover (r = 0.590, $p < 0.05$), forage yield (r = 0.534, $p < 0.05$), and seed yield (r = 0.526, $p < 0.05$). However, the accessions collected from high elevations (960 up to 2040 masl) seemed to be more tolerant to cold than the accessions collected from low elevations (Table 4). This finding was in conformity with some previous reports on annual medics (Cocks and Ehrman 1987; Yahia and Fyad-Lameche 2003; Yahia et al. 2014). Furthermore, tolerance to cold varied among species.

Finally, it can be interpreted from results of Table 4 and cluster and factor analyses (Figures 2 and 3) that the order of cold tolerance was *M. rigiduloides* > *M. rigidula* > *M. minima* > *M. noeana*. Therefore, we recommend that accessions 51TN01851, 51TN01853, 51TN01842, 51TN01048, 51TN01844, and 51TN00787 be used in the breeding programs. As these accessions were highly cold-tolerant and produced high biomass, they can be used to replace *M. scutellata* in the southwestern Iran or in other regions of the world with similar climate. As there were high levels of diversity for most of the evaluated traits within and among species, next step of the research should be utilization of the existing diversity for relevant purposes (such as screening for biotic and abiotic stresses) or to apply them in annual medics breeding programs to develop and release varieties with required traits for specific regions.

Disclosure statement

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References

Abbasi, M. R. 2015. "Evaluation of Iranian Wild *Medicago* Genetic Resources in order to Use in Agronomic Systems." *Electronic Journal of Crop Production* 8 (3): 225–238.

Abbasi, M. R., A. Mirakhorli, A. Mahdipur, A. Hasanzadeh, R. Kanani, R. A. Alitabar, H. Mokhtarpour, H. Safaei, A. Fathi, and G. H. Khakizad. 2014. "Iranian *Medicago* Genetic Resources: Exploration, Collection, Conservation and Centers of Diversity." *Agronomy Journal (Pajouhesh & Sazandegi)*, 28 (106): 55–63.

Abd El-Moneim, A. M., and P. S. Cocks. 1986. "Adaptation of Medicago Rigidula to a Cereal-Pasture Rotation in North-West Syria." *The Journal of Agricultural Science* 107 (01): 179–186. doi:10.1017/S0021859600066934.

Alizadeh, K., and B. Sadeghzadeh. 2010. "Cold Tolerance of Iranian Annual Medic Species under Cold Dryland Condition." *Journal of Food, Agriculture & Environment* 8 (2): 386–390.

Angus, J. F., and M. B. Peoples. 2013. "Nitrogen from Australian Dryland Pastures." *Crop and Pasture Science* 63 (9): 746–758. doi:10.1071/CP12161.

Caddel, J. 2011. "Potential for Rigid Medics in the Southern Plains Medicago Rigidula (L.) All. And Medicago Rigiduloides E. Small." Accessed 11 July. http://forage.okstate.edu/rigidmedics/rigid_medics.htm

Cocks, P. S., and T. A. M. Ehrman. 1987. "The Geographic Origin of Frost Tolerance in Syrian Pasture Legumes." *Journal of Applied Ecology* 24 (2): 673–683. doi:10.2307/2403902.

Crawford, E. J., A. W. H. Lake, and K. G. Boyce. 1989. Breeding annual Medicago species for semiarid conditions in southern Australia. In: Advances in agronomy (Edited by N.. Brady; Published by Academic Press Inc), Vol: 42: 399–437. https://books.google.com/books?hl=en&lr=&id=cHap6AaWrPQC&oi=fnd&pg=PA399&dq=Breeding+annual+Medicago+species+for+semiarid+conditions+in+southern++Australia&ots=Ys0r8g54sj&sig=PmyQrwbK2blloVZmeIxSg_DU3bI#v=onepage&q&f=false

Dehaghi, M. A., and S. A. M. M. Sanavy. 2003. "Effect of Root-Zone Temperatures on Morphology, Growth and Development, Yield, and Yield Components of Annual Medics." *Australian Journal of Agricultural Research* 54 (9): 917–921. doi:10.1071/AR02067.

Dori, M. A. 2008. "Forage Production of Eight Annual Medic Cultivars under Rainfed Conditions of Gollestan Province." *Journal of Agricultural Science and Technology* 10 (2): 185–190.

Entz, M. H., V. S. Baron, P. M. Carr, D. W. Meyer, S. Smith, and W. Paul McCaughey. 2002. "Potential of Forages to Diversify Cropping Systems in the Northern Great Plains." *Agronomy Journal* 94 (2): 240–250. doi:10.2134/agronj2002.0240.

Hekneby, M., M. C. Antolín, and M. Sánchez-Díaz. 2006. "Frost Resistance and Biochemical Changes during Cold Acclimation in Different Annual Legumes." *Environmental and Experimental Botany* 55 (3): 305–314. doi:10.1016/j.envexpbot.2004.11.010.

IPGRI. 1991. *Annual Medic Descriptors*. Rome, Italy: International Plant Genetic Resources Institute.

James, F. C., and C. E. McCulloch. 1990. "Multivariate Analysis in Ecology and Systematics: Panacea or Pandora's Box?" *Annual Review of Ecology and Systematics* 21 (1): 129–166. doi:10.1146/annurev.es.21.110190.001021.

Krall, J., R. W. Groose, and J. Sobels. 1996. Winter Survival of Austrian Winter Pea and Annual Medic on the Western High Plains. Paper presented at the Progress in New Crops, Alexandria, VA.

Leilah, A. A., and S. A. Al-Khateeb. 2005. "Statistical Analysis of Wheat Yield under Drought Conditions." *Journal of Arid Environments* 61 (3): 483–496. doi:10.1016/j.jaridenv.2004.10.011.

Lesins, K. A., and I. Lesins. 2012. *Genus Medicago (Leguminosae): A Taxogenetic Study*. Dordrecht, Netherland: Springer Science & Business Media.

Romesburg, C. 2004. *Cluster Analysis for Researchers*. (Lulu. com.) North Carolina, USA: Lulu Press

Tork Nejad, A. 1998. *Ecological Potential of Iranian Annual Medics*. Tehran, Iran: Tarbiat Modarres University.

Walsh, M. J., R. H. Delaney, R. W. Groose, and J. M. Krall. 2001. "Performance of Annual Medic Species (Spp.) In Southeastern Wyoming." *Agronomy Journal* 93 (6): 1249–1256. doi:10.2134/agronj2001.1249.

Yahia, N., and F. Z. Fyad-Lameche. 2003. "Evaluation de la variabilité de jeunes plants de *Medicago* soumis à un régime de basse température." *Acta botanica gallica* 150 (1): 3–17. doi:10.1080/12538078.2003.10515983.

Yahia, N., F. Lameche, Z. Fatima, N. Bakhti, and P. Barre. 2014. "Characterization of *Medicago* Populations under Cold Acclimation by Morphological Traits and Microsatellite (SSR) Markers." *African Journal of Biotechnology* 13 (27): 2704–2714. doi:10.5897/AJB.