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Forage Yield in some Iranian Wild *Trifolium* Genetic Resources under Different Climatic and Irrigation Conditions

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ABSTRACT

Clovers (Trifolium spp.) are one of the main forage crops in temperate regions. This research was conducted to identify water-stressed tolerance among Iranian wild annual clovers under different climatic regions. Seventeen accessions, belonging to seven species, were planted in a field in two consecutive years, at two locations (Mashhad and Urmia) in Iran, under normal and water-stressed conditions. Combined analysis of variance, Scheffe analysis, and Duncan's new multiple range test showed significant differences in forage production among clover accessions as well as species. In addition, drought-tolerance/susceptibility indices were calculated for each accession and species. Then, these indices were applied in factor analysis. Extracted Bi-plot based on factor analysis confirmed the results of Duncan and Scheffe analyses. In water-stressed conditions, T. resupinatum (cultivated accession) produced the highest forage in Urmia with 349 mm annual rainfall, whereas in Mashhad, with 149.8 mm annual precipitation, T. purpureum and T. echinatum (both, wild species) had the highest production. The accessions as well as species were ranked based on their forage production for each location and experiment. Ultimately, this work proposed some new Trifolium species, such as T. echinatum, T. diffusum, and T. purpureum, for forage production in agronomic systems.

Keywords: Annual clovers, Drought tolerance/susceptibility indices, Mashhad, Urmia.

INTRODUCTION

Clovers (*Trifolium* spp.) are one of the main forage crops in temperate regions, especially in Mediterranean climate zones. More than 50 *Trifolium* species are naturally distributed in Iran (Rechinger, 1984; Mozaffarian, 1998; Abbasi, 2009; Haerinasab and Rahiminejad, 2012). *Trifolium resupinatum* (Persian clover) is the main clover species in arable lands of the country with a cultivation area of about 60,000 ha (Abbasi, 2008b; Abbasi and Zamanian, 2008). Other *Trifolium* species, although widely distributed across the country (Rechinger, 1984; Mozaffarian, 1998; Haerinasab and Rahiminejad, 2012), are rarely used for forage production, except in rangelands,

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which are naturally mixed with other legumes or grasses. Red clover (*T. pratense* L.) and berseem clover (*T. alexanderinum* L., not indigenous species) are cultivated in a limited area (17,000 ha) (Pourtaghi *et al*., 2005; Abbasi, 2008a; Zamanian *et al*., 2008). Most of the *T. resupinatum* seeds in the country are landraces, which have been selected by farmers across time from the wild parents. Also, there are a few breeding lines that have been released recently (Abbasi and Zamanian, 2008).

Annual clovers are used for forage production in other parts of the world, including Australia (Nichols *et al*., 2010). Morris and Greene (2001) listed 16 clover species that are cultivated worldwide. There are some other wild annual species with high production in Iran, including *T. purpureum*, *T. echinatum* , and *T. diffusum* (Abbasi *et al*., 2011), which have not been mentioned by Morris and Greene (2001). Former researches in Iran have shown that *T. purpureum*., *T. echinatum*, *T. diffusum*, *T. hirtum*, and *T. lappaceum* produce suitable forage yield and have other good agronomic traits (Abbasi, 2009; Abbasi *et al*., 2011). Therefore, these wild species could be potential candidates to be introduced to agrosystems.

Several selection indices based on mathematical relationships have been developed to identify plant tolerance to stress such as Stress Tolerance Index (STI) and stress susceptibility index. These indices have been largely used to identify germplasms' susceptibility in different crops (Nikou *et al*., 2014; Abbasi *et al*., 2018; Mortazavian *et al*., 2018). Some studies (Abdolshahi *et al*., 2013; Sardouie-Nasab *et al*., 2014) have applied multivariate analyses on the indices to identify salt- or drought-tolerant genotypes. However, there is no report that deals with drought-tolerance/susceptibility indices as well as multivariate analysis on *Trifolium* species studied in this study.

In Iran, clover species are usually cultivated in regions with suitable natural precipitation as autumn crop. However, there is a limitation for the irrigation in the spring. This is because of the competition for the irrigation among clover and other crops such as cereals and vegetables. Annual forage crops that could tolerate water deficit in the spring could be a good candidate for using in the agrosystems with low water resources. Hence, objective of this research was to find some annual wild clovers, selected from former investigations (Abbasi, 2008b; Abbasi, 2009; Abbasi *et al*., 2011; Abbasi *et al*., 2012), which can produce high amount of forage, the same as cultivated landraces, under water-stressed conditions.

MATERIALS AND METHODS

Plant Materials and Experimental Field

Seventeen superior accessions of *Trifolium* species selected from former researches (Abbasi, 2008b; Abbasi, 2009; Abbasi *et al*., 2011; Abbasi *et al*., 2012) were used as plant materials in the study (Table 1). These seeds are conserved in National Plant Gene Bank of Iran. They were planted in an experimental field on September 5, 2010, and sown again in September 2011, in a randomized complete-block design with three replications, at two locations: Mashhad (36° 16´ 15´´ N, 59° 38´ 30´´ E, 999 m) and Urmia (37° 85´ 04´´ N, 45° 02´ 33´´, 1328 m), and in two experiments (normal and water-stressed conditions). Each accession was planted on a plot size of 2×3 m with four rows having three m lengths and 0.5 m apart from each other. There was 1 m distance between 2 blocks, 5 m between the two experiments. Irrigation was performed from planting time (September) to November, until fall precipitation, with eight days intervals, in both experiments. From November to mid-April, no irrigation was performed because of natural precipitation. From mid-April to harvesting time (end of May), there were two types of irrigation: normal irrigation experiment (normal) with eight days intervals, and water-stressed experiment (stress) with 16 days intervals.

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Table 1. Species, accession number, and origin of annual clover germplasms.

Row	Accession number	Species	Province	Type of collection	Altitude (m)
1	50TN00592	*T. echinatum*	Kermanshah	wild	500
2	50TN00638	*T. campestre*	Fars	wild	970
3	50TN00708	*T. echinatum*	Azarbaijan Sharghi	wild	1340
4	50TN00756	*T. hirtum*	Lorestan	wild	1450
5	50TN00775	*T. resupinatum*	Kordestan	cultivated	1050
6	50TN00822	*T. diffusum*	Azarbaijan Gharbi	wild	1520
7	50TN00864	*T. echinatum*	Azarbaijan Gharbi	wild	1220
8	50TN01238	*T. lapaceum*	Kermanshah	wild	600
9	50TN01240	*T. campestre*	Kermanshah	wild	600
10	50TN01293	*T. purpureum*	Kermanshah	wild	1150
11	50TN01310	*T. purpureum*	Kermanshah	wild	1200
12	50TN01337	*T. purpureum*	Kermanshah	wild	700
13	50TN01443	*T. diffusum*	Azarbaijan Gharbi	wild	1220
14	50TN01444	*T. diffusum*	Azarbaijan Gharbi	wild	No data
15	50TN01514	*T. resupinatum*	Fars	cultivated	No data
16	50TN01586	*T. purpureum*	Kermanshah	wild	1100
17	50TN01794	*T. resupinatum*	Mazandaran	wild	440

No fertilizers and chemicals were used during this study. Meteorological data were downloaded from the Khorassan-e Razavi and Azarbaijan-e Gharbi Climatic Data Center's Network Portal, the climatological sites near the experimental fields (Table 2).

Forage yield was evaluated according to IPGRI descriptors (IPGRI, 1984). In each plot, a 1×2.5 m section was harvested to a stubble height of 3-5 cm with sickle. To determinate dry matter yield, a sub-sample (500 g) of each plot was dried in an electric oven at 60°C for 72 hours.

Stress-tolerance or susceptibility indices were calculated using the following expressions:

$$SSI(\text{stress susceptibility index}) = \frac{1-\left[\frac{[Ys]}{Yp}\right]}{SI}$$

$$SI(\text{susceptibility index}) = 1-\left[\frac{\overline{YS}}{\overline{YP}}\right]$$

,

(Fischer and Maurer, 1978)

$$STI(\text{stress tolerance index}) = \frac{(Yp)(Ys)}{\overline{YP}^2}$$

(Fernandez, 1992)

TOL (Tolerance)= Yp –Ys (Hossain *et al*., 1990)

MP (Mean Productivity)= (Yp+Ys)/2 (Hossain *et al*., 1990)

GMP (Geometric Mean Productivity)= (Yp×Ys)^0.5 (Fernandez, 1992)

HM (Harmonic Mean) = 2(Ys×Yp)/(Ys+Yp) (Rosielle and Hamblin, 1981)

Where, *Ys* and *Yp* are dry Yield of each accession under stress and non-stress conditions, $\overline{Ys}$ and $\overline{Yp}$ are the mean dry Yield of all accessions under stress and control conditions, respectively.

Data Analysis

Analysis of variance, Scheffe analysis, and Duncan's new multiple range test were performed on data. Data were transformed by square root. In combined analysis of variance, randomized effects were considered for year, experiment, and location; while fixed ones for accession (Yazdi Samadi *et al*., 1997).

Factor analysis was performed on drought-tolerance/susceptibility indices to predict accession behavior in the stress conditions. In this analysis, extraction method was principal component analysis

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Table 2. Annual precipitation (mm) in Mashhad and Urmia during the two study years.

Month	Mashhad 1st Year	Mashhad 2nd Year	Urmia 1st Year	Urmia 2nd Year
September	21.2	2.4	30.5	0.1
October	0	0.5	0	3.6
November	5.8	8.6	79.4	8.2
December	20.8	0	19.7	5.2
January	3.7	13.1	15.3	36.1
February	18.2	33.5	12	10.6
March	51.4	23.6	71.8	80.6
April	27.6	11.1	48.5	46.1
May	37.4	15.9	109.7	112.8
June	5	0	4.1	3.7
Sum	191.1	108.7	391	307
Mean of 2 years	149.8		349	
Mean of March to May	83.5		234.7	

and rotation method was Varimax with Kaiser Normalization. The statistics of Factor (F) were calculated. Then, Bi-plot was extracted based on two factors of the factor analysis. Pearson's correlation analysis was used to calculate correlation coefficient among *F* and drought-tolerance/susceptibility indices. Data analyses were performed by MSTAT-C and SPSS 15.

RESULTS

Table 2 shows the amount of annual precipitation in both locations. Urmia had more rainfall than Mashhad in both years. All species germinated in the field, although the cultivated *T. resupinatum* germinated sooner than other species. These accessions also had good growth compared with wild species in autumn. Nevertheless, in the spring, all species covered thoroughly the plot area because of their fast vegetative regrowth; however, there were differences in regrowth rate among species.

Table 3 shows the results of combined analysis of variance for dry forage yield among accessions, which properly indicated the effects of year, accessions, locations, and their interactions on forage production. Scheffe analysis grouped species in each

Table 3. Produced *F* values from combined analysis of variance for dry forage yield in the both locations and experiments.

Sources of variations	*df*	*F*, Stress	*F*, Normal	*F*, Urmia	*F*, Mashhad
Year	1	55.71^{ns}	0.006^{ns}	0.015^{ns}	599.36**
Experiment (or Location)	1	176.76*	61.29^{ns}	22.274^{ns}	134.67^{ns}
Year×Experiment (or Location)	1	0.055^{ns}	4.31^{ns}	4.371^{ns}	0.0029^{ns}
Year×Experiment (or Location)× Block	8				
Accessions	16	9.44**	12.88**	10.057**	5.63**
Accessions×Year	16	0.682^{ns}	0.97^{ns}	2.315^{ns}	2.61*
Accessions×Experiment (or Location)	16	3.272*	6.41*	2.016^{ns}	0.489^{ns}
Accessions×Experiment (or Location)×Year	16	1.714^{ns}	1.061^{ns}	0.695^{ns}	0.911^{ns}
Error	128				

*: Significant at 0.05; **: Significant at 0.01, ns: Not significant.

location and expriment (Table 4). In the normal irrigation experiment in Mashhad, *T. purpureum, T. echinatum,* and *T. hirtum with* a mean of 8.05 to 8.50 t ha^{-1} dry forage yield were located at the highest level, whereas, in the normal experiment in Urmia, *T. resupinatum* (the cultivated one) produced the highest yield with a mean of 23.92 t ha^{-1}. In stress experiment in Mashhad, *T. echinatum* and *T. purpureum* showed the highest production with a mean of 7.77 to 7.87 t ha^{-1}, whereas in Urmia, *T. resupinatum* (the cultivated accessions with a mean of 13.7 t ha^{-1}) yielded the most (Table 4).

Table 5 shows dry forage yield among accessions in each location. In Mashhad in the normal experiment, accessions 50TN01310 and 50TN01586, belonging to *T. purpureum,* showed the highest

Table 4. The grouping of annual clover species (based on Scheffe analysis) for mean of dry forage yield (t ha^{-1}).[a]

Species	Number of replications	Mashhad Normal	Mashhad Stress	Urmia Normal	Urmia Stress
T. campestre	12	3.65[a]	3.70[a]	3.32[a]	2.50[a]
T. diffusum	18	7.47[bc]	6.97[bc]	13.47[bc]	6.87[abc]
T. echinatum	18	8.05[c]	7.77[c]	18.20[bcd]	13.07[bc]
T. hirtum	6	8.50[c]	7.00[bc]	12.85[ab]	6.02[ab]
T. lappaceum	6	6.07[bc]	5.67[abc]	17.45[bcd]	11.00[bc]
T. purpureum	24	8.12[c]	7.87[c]	14.05[bcd]	7.72[abc]
T. resupinatum y (Cultivated)	12	4.85[ab]	4.55[ab]	23.92[d]	13.7[c]
T. resupinatum (Wild*)*	6	6.07[bc]	5.40[abc]	23.90[cd]	11.60[bc]

[a] In each column, different letters indicate significant differences.

Table 5. The grouping of annual clover accessions (in Duncan test) for mean of dry forage yield (t ha^{-1}).[a]

Species	Accession number	Number of replications	Mashhad		Urmia	
			Normal	Stress	Normal	Stress
T. echinatum	50TN00592	6	8.20[def]	8.20[efg]	14.92[bcd]	14.57[fg]
T. campestre	50TN00638	6	3.25[a]	4.12[ab]	3.20[a]	3.25[ab]
T. echinatum	50TN00708	6	8.27[ef]	8.57[fg]	17.30[bcdef]	10.67[cdef]
T. hirtum	50TN00756	6	8.50[ef]	7.00[cdefg]	12.85[b]	6.00[bcd]
T. resupinatum (cultivated)	50TN00775	6	5.17[bc]	4.82[abc]	26.15[f]	19.60[g]
T. diffusum	50TN00822	6	8.22[def]	6.92[cdefg]	16.97[bcde]	8.00[cde]
T. echinatum	50TN00864	6	7.67[def]	6.62[cdef]	22.80[def]	14.15[fg]
T. lapaceum	50TN01238	6	6.07[cd]	5.67[bcde]	17.45[bcdef]	11.00[def]
T. campestre	50TN01240	6	3.77[ab]	3.30[a]	3.45[a]	1.82[a]
T. purpureum	50TN01293	6	7.45[def]	7.32[defg]	17.15[bcdef]	7.07[bcde]
T. purpureum	50TN01310	6	8.70[f]	9.45[g]	15.20[bcd]	7.17[bcde]
T. purpureum	50TN01337	6	7.70[def]	6.65[cdef]	10.03[b]	7.92[cde]
T. diffusum	50TN01443	6	7.92[def]	7.60[defg]	12.75[b]	5.60[bc]
T. diffusum	50TN01444	6	6.32[cde]	6.42[cdef]	11.05[b]	7.17[bcde]
T. resupinatum (cultivated*)*	50TN01514	6	4.55[abc]	2.27[ab]	21.82[cdef]	8.85[cdef]
T. purpureum	50TN01586	6	8.72[f]	8.20[efg]	13.95[bc]	9.75[cdef]
T. resupinatum (wild)	50TN01794	6	6.07[cd]	5.40[bcd]	23.90[ef]	11.60[ef]

[a] In each column, different letters indicate significant differences.

production with a mean of 8.7 to 8.72 t ha^{-1}, whereas in Urmia, 50TN00775, the cultivated accession of *T. resupinatum*, produced the highest yield with a mean of 26.15 t ha^{-1} (Table 5). The accessions 50TN01310 and 50TN00775 produced the highest dry forage in the stress experiment in Mashhad and Urmia, respectively.

Result of factor analysis revealed that, in both locations, *MP*, *STI*, *GMP* and *HM* indices had the highest effect on the first Factor (F1) (Table 6), while the second factor showed high communalities with *SSI* and *TOL* indices. Therefore, F1 indicated drought-tolerance property of the accessions and those accessions that received higher scores for this factor were the more tolerant ones. Similarly, the second factor was assumed as susceptible one identifying low-yielding and highly drought-susceptible accessions with greater values for *SSI* and *TOL* (Table 6). Drought-tolerance indices (STI, MP, GMP, and HM) showed highly significant correlation with F1 statistics, while the susceptible ones (SSI and TOL) correlated with F2 statistics (Table 7). Hence, F1 is categorized as tolerant and F2 as susceptible statistics. Extracted Bi-plot based on the two first factors scattered accessions (Figure 1) as well as species (Figure 2) regarding their stress-susceptibility. Accessions or species on part II of the Bi-plot were the most susceptible, and the ones on part IV were the most

Table 6. Results of factor analysis on drought-tolerance/susceptibility indices in two locations.[a]

	Factors			
	Mashhad		Urmia	
Variables	1	2	1	2
Total eigenvalues	3.98	1.95	4.09	1.81
Percent of variance	66.4	32.5	68.2	30.1
The cumulative percent of variance	66.4	98.9	68.2	98.3
	Eigenvectors			
STI (Stress Tolerance index)	0.996	-0.036	0.981	-0.049
SSI (Stress Susceptibility Index)	0.023	0.986	-0.215	0.964
MP (Mean Productivity)	0.999	0.019	0.987	0.143
GMP (Geometric Mean Productivity)	0.998	0.044	0.995	0.076
TOL (Tolerance)	0.003	0.986	0.350	0.922
HM (Harmonic Mean)	0.999	0.026	0.998	0.006

[a] Extraction Method: Principal Component Analysis, Rotation Method: Varimax with Kaiser Normalization.

Table 7. Pearson correlation coefficients between tolerance/susceptibility indices and F statistics in Mashhad (above diagonal) and Urmia (below diagonal).

Indices	*STI* [a]	*SSI* [b]	*MP* [c]	*GMP* [d]	*TOL* [e]	*HM* [f]	*F1* [g]	*F2* [h]
STI [a]		-0.011	0.994**	0.989**	-0.032	0.992**	0.996**	-0.036
SSI [b]	-0.248		0.04	0.066	0.944**	0.049	0.023	0.986**
MP [c]	0.950**	-0.076		0.997**	0.024	0.999**	0.999**	0.019
GMP [d]	0.963**	-0.14	0.997**		0.046	0.999**	0.998**	0.044
TOL [e]	0.291	0.787**	0.478	0.416		0.028	0.003	0.986**
HM [f]	0.970**	-0.205	0.989**	0.997**	0.35		0.999**	0.026
F1 [g]	0.981**	-0.215	0.987**	0.995**	0.35	0.998**		0
F2 [h]	-0.049	0.964**	0.143	0.076	0.922**	0.006	0	

[a] Stress Tolerance Index, [b] Stress Susceptibility Index, [c] Mean Productivity, [d] Geometric Mean Productivity, [e] Tolerance, [f] Harmonic Mean , [g] Factor 1 in factor analysis, [h] Factor 2 in factor analysis, **: Significant at 0.01.

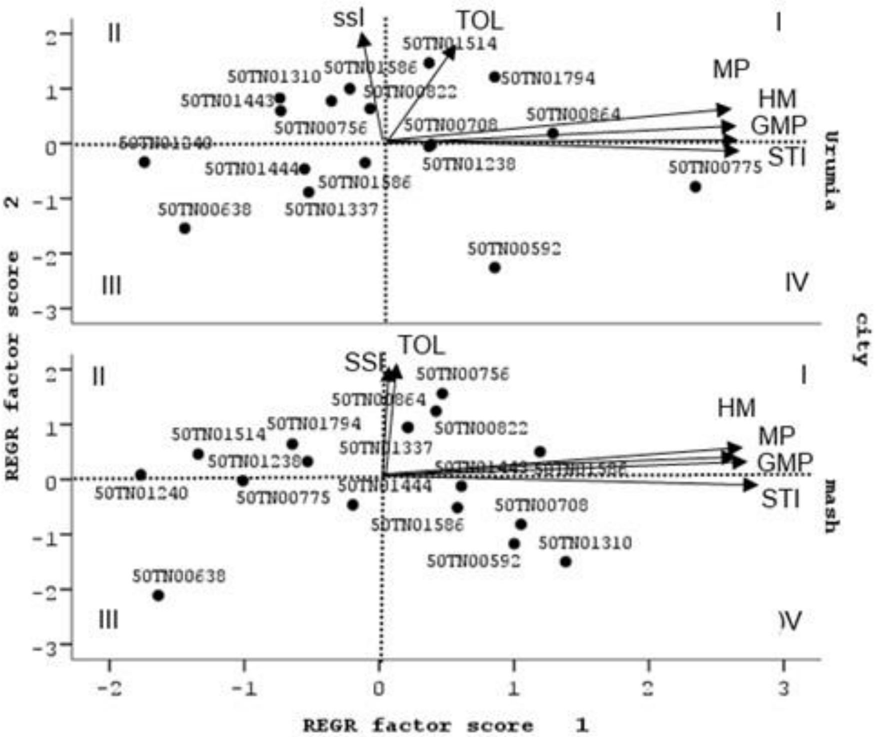

Figure 1. Distribution of *Trifolium* accessions in the Bi-plot based on two first factors of factor analysis in two locations; English figures represent the accession number (Table 1) and Greek figures show the Bi-plot parts; arrows indicate susceptibility/tolerance indices.

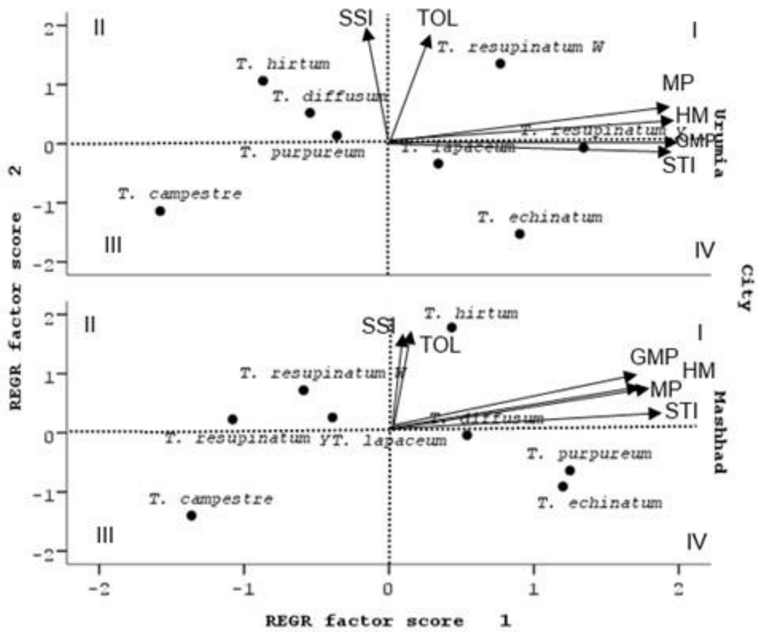

Figure 2. Distribution of *Trifolium* species in the Bi-plot based on two first factors of factor analysis in two locations; Greek figures show the Bi-plot parts, arrows represent susceptibility/tolerance indices.

tolerant. Also, accessions or species on part I of the Bi-plot were moderately tolerant and on part III were low-yielding tolerant (Figures 1 and 2).

DISCUSSION

Cultivation of *T. resupinatum* has a long history in Iran and many landraces have been developed for high forage production by farmers (Abbasi, 2008b; Abbasi and Zamanian, 2008). *T. resupinatum*, mainly in its multi-cut types, is sensitive to water stress when the temperature begins to increase and precipitation gradually decreases from middle of spring onwards (Abbasi and Zamanian, 2008). In this case, a significant decrease occurs in forage production of cultivated *T. resupinatum* if irrigation intervals are delayed 2-3 days in comparison with normal irrigation intervals (7-8 days). This phenomenon usually takes place in early spring because of the increasing demand for irrigation in other crops (Abbasi and Zamanian, 2008). Therefore, in the case of deficiency in natural precipitation, forage yield of *T. resupinatum* landraces will be affected severely. Hence, one of the main duties in our Plant Gene Bank is to identify suitable clover germplasms able to tolerate different water stress regimes. These materials could be a reasonable candidate to be replaced by *T. resupinatum* landraces in some regions with deficiency in precipitation. Therefore, this study could identify some Iranian wild annual clovers that are more tolerant to water-stress conditions than the cultivated ones (*T. resupinatum*).

The studied clover accessions showed different responses at two different locations in two consecutive years (Table 3). The reason that the year effect was significant in Mashhad, in contrast to Urmia, was the precipitation amount, mainly in the early spring (Table 2). In other words, if the annual precipitation is about 349 mm or in early spring is near 234.7 mm (Table 2), the year effect will not be significant. In addition, accession × year effect was significant in Mashhad in contrast to Urmia. Therefore, these results revealed that precipitation amount has a significant effect on forage production of the annual clovers.

T. purpureum and *T. echinatum* in Mashhad and the cultivated *T. resupinatum* in Urmia produced the highest yields in both experiments and in both years (Table 4). In Urmia with sufficient precipitation (Table 2), *T. resupinatum* produced the highest yield. However, in Mashhad with insufficient precipitation, *T. purpureum* and *T. echinatum*, wild species, showed the highest forage production. These findings indicated that wild species in contrast to cultivated ones had stable production in the regions with low precipitation (Table 4). However, when water was not a limiting factor, as it happened in Urmia, the cultivated species showed the highest forage production. This finding also indicated that domestication of *T. resupinatum* by farmers has been a proper selection in Urmia and in the country. Of course, in the case of *T. campestre*, because of its small size, it produced the lowest yield in all conditions and locations (Table 4). The results of Scheffe analysis (Table 4) was in accordance with Bi-plot results (Figure 2). Regarding this, *T. purpureum* was located at the highest level (c level) by Sheffe analysis with a mean of 7.87 and 8.12 t ha^{-1} dry forage yield in Mashhad, in both experiments. This species was located at the part IV of the Bi-plot (Figure 2). In Urmia, *T. resupinatum* y (the cultivated accession) appeared at the highest level of Scheffe analysis (Table 4) and in the Bi-plot it appeared at part IV too (Figure 2). According to Dunccan analysis (Table 5), accessions 50TN01310 and 50TN01586 produced the highest dry forage yield in Mashhad in both experiments, while in Urmia, accession 50TN00775 had the highest production. These accessions appeared at part IV of bi-plat too (Figure 1). Our results from factor analysis were also in accordance with Abdolshahi *et al*., (2013) and Sardouie-Nasab *et al*. (2014) findings.

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That the wild clovers, mainly *T. purpureum* and *T. echinatum*, produced more forage than cultivated one in Mashhad (with dry temperate climate) could be explained by differences in morphological traits (such as leaf or stem hairiness). In other words, the wild species showed much more hairiness on their leaves and stems than the cultivated ones (*T. resupinatum*) (Abbasi 2008b, 2009). This characteristic has a major role in harsh and dry climates to reduce water loss in plants (Espigares and Peco, 1995).

Annual legumes have been used to extend the length of the grazing season, improve nutritional quality of herbage, and reduce usage of nitrogen fertilizer (Kuusela *et al*., 2004; Rao *et al*., 2005; Kelly *et al*., 2006). Sixteen major taxa of annual clovers that are used as forage have been presented in a review by Morris and Green (2001). These species are cultivated in many countries such as *T. alexanderinum* in Egypt (Hassan *et al*., 2007), *T. hirtum*, *T. michelianum*, *T. glanduliferum*, *T. purpureum*, *T. spumosum* L., and *T. subterraneum* in Australia (Dear *et al*., 2002; Nichols *et al*., 2010; Loi *et al*., 2012). Besides these species, we reported that there are good forage production in *T. echinatum*, *T. diffusum* and T. *purpureum* (Table 4) to use for forage production for some parts of Iran and similar climatic regions across the world. Of course, there is a report that mentions the use of *T. purpureum* as forage crop in Australia with a dry forage production in excess of 10 t ha^{-1} (Nichols *et al*., 2010), which is in accordance with our research (Table 4).

Clovers naturally grow in the regions with adequate water resources or cultivated in irrigated farms (Taylor, 1985). Most researches on drought tolerance in *Trifolium* species have been performed on *T. repens*, a perennial species (Jiang *et al*., 2010; Li *et al*., 2013; Vaseva *et al*., 2014) and some of them on *T. pratense*, another perennial kind (Vaseva *et al*., 2014). However, there were a few researches on study of drought tolerance in annual clovers (Fraser *et al*., 2004; McCartney and Fraser, 2010). Hence, our work has provided new findings concerning some annual clovers responses, particularly *T. diffusum*, *T. echinatum*, and *T. purpureum*, to water-stressed conditions. Therefore, these findings can be used to release suitable cultivars in breeding programs.

Further investigations are needed to identify molecular mechanisms of tolerance to water stress in the studied species. In the case of *T. repens*, it has been proved that low molecular proteins (identified as nucleoside di-phosphate kinase and lipid transfer proteins) (Lee *et al*., 2014) or pinitol, the major soluble sugar present in mature leaves, (McManus *et al*., 2000) play a role in response to water-deficiency stress. Meanwhile, the studied species can be used in breeding programs to release some tolerant hybrids as it has been performed for *T. repens* by Nichols *et al*. (2013). They have improved drought stress tolerance in white clover through hybridization with *T. uniflorum.* In our evaluated materials, *T. diffusum* is secondary gene pool for *T. pretense* (Morris and Greene, 2001).

CONCLUSIONS

Annual precipitation has a key role in forage production of annual clovers. Based on this study, it can be stated that in Mashhad, in descending order, *T. purpureum*, *T. echinatum*, *T. hirtum*, *T. diffusum*, *T. lappaceum* and *T. resupinatum* (wild) produced the highest forage and showed high tolerance to water stress, whereas in Urmia, the order was *T. resupinatum*, *T. echinatum*, *T. lappaceum*, *T. purpureum* and *T. diffusum*. In the case of accessions in Mashhad, it was 50TN01310, 50TN00708, 50TN01586, and 50 TN00592, and in Urmia, it was 50TN00775, 50TN00592, 50TN000864, and 50TN01794. These results show cultivated Trifolium species (*T. resupinatum*) is suitable species for humid temperate regions with high rainfall such as Urmia region. Nevertheless, in Mashhad, with dry temperate climate, the wild annual clovers are more suitable and produce more forage in water-stressed conditions. Furthermore, some wild annual clover species such as *T. echinatum*, *T. diffusum*, and *T. purpureum* were proposed to use for high forage production (in addition to Green and Morris (2001) report) that can be used to release suitable cultivars in breeding programs.

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ارزیابی عملکرد علوفه در برخی از منابع ژنتیکی شبدرهای وحشی ایران در شرایط آبی و اقلیمی مختلف

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چکیده

شبدرها (*Trifolium* spp.) یکی از گیاهان علوفه‌ای مهم در مناطق معتدله هستند. در این تحقیق تحمل به تنش آبی در منابع ژنتیکی شبدرهای یک‌ساله وحشی ایران در مناطق اقلیمی مختلف بررسی گردید. هفده توده متعلق به هفت گونه در یک مزرعه آزمایشی در دو منطقه (مشهد و ارومیه) طی دو سال در دو شرایط آبی نرمال و تنش مورد آزمایش قرار گرفتند. تجزیه مرکب، آزمون‌های شفه و دانکن اختلاف معنی داری را برای علوفه خشک در بین توده‌ها و گونه‌ها نشان دادند. همچنین شاخص‌های حساسیت پذیری و تحمل به خشکی در گونه‌ها و توده‌ها محاسبه شدند. سپس این شاخص‌ها در تجزیه به عامل‌ها استفاده شدند. بای‌پلات حاصله یر اساس دو عامل اول، نتایج حاصل از آزمونه‌های دانکن و شفه را تایید نمود. در شرایط تنش آبی، توده‌های زراعی گونه *T. resupinatum* بیشترین عملکرد را در ارومیه (با ۳۴۹ mm بارش سالانه) داشت در صورتی که در مشهد (با ۱۴۹/۸ mm بارش سالانه) گونه‌های وحشی *T. purpureum* و *T. echinatum* بیشترین تولید را داشتند. گونه‌ها و توده‌ها بر اساس تولید علوفه‌شان در مناطق و شرایط تنشی مورد آزمایش رتبه بندی شدند. در نهایت، این تحقیق گونه‌های جدیدی از شبدر همانند *T. echinatum*، *T. diffusum* و *T. purpureum* برای تولید علوفه در سیستم‌های زراعی را معرفی نمود.