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Seasonal changes in some physiological and biochemical responses of six groundcover plants

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Abstract
Landscape groundcover plants are a diverse group of trailing or spreading species that naturally form continuous soil coverage. Ground cover plants that were used in this study consisted of vegetative propagules of: *Vinca minor* L. 'Variegatum', *Vinca minor* L. 'Green', *Oxalis brasiliensis* G. Lodd, *Trifolium repens* L., *Phyla nodiflora* L. and *Frankenia thymifolia* Desf. The aim of this study was to compare some physiological and biochemical responses of each species used under seasonal changes in Shiraz climatic conditions. Several factors including chlorophyll and relative water content (RWC), electrolyte leakage (EL), proline content, soluble sugars and starch levels and antioxidant enzyme activities of these ground covers were investigated. Results showed that chlorophyll and RWC content were decreased in all ground cover plants during summer time. Furthermore, considerable seasonal variations in antioxidant enzyme activities were detected in all of the studied plants. The highest activity of CAT was obtained in summer, while SOD, POD and APX had their highest activities in autumn and winter seasons. Proline concentration in *F. thymifolia* was higher than the other species, especially in mid-winter. According to the results of this research, it appears that plants show different mechanisms against natural adverse environmental conditions. It can be concluded that *F. thymifolia and P. nodiflora* are well-matched with warm season and *T. repens* and *V. minor* are well-matched with cool season environmental conditions. Therefore, these plants can be recommended for cultivation in Shiraz green space and the same conditions elsewhere.

Keywords: Establishment, Antioxidant enzymes, Seasonal change.

Introduction

Ground cover plants can be well suited alternatives for turfgrasses to overcome some of the challenges for turfgrass culture e.g., water shortage and plant maintenance. Ground cover plants may be described as those which tend to grow horizontally rather than upright and have a dense profusion of leaves. They typically range in height from 7.5 cm to nearly 1.0 m tall and may be woody, herbaceous, or succulent (Pittenger et al., 2001). The use of ground covers within the urban environment not only helps to cool down the environment, but will also boost performance of other landscape plants by lowering air, soil and surface temperatures, raising the relative humidity and reducing air vapor pressure deficits better than concrete and asphalt surfaces (Montague et al., 2000). Establishment of appropriate ground covers that can be sustained with no maintenance or periodic mowing is preferred (Weston, 2002). Ghani et al. (2011) investigated six *Achillea* species as ground cover plants and found that *A. filipendulina*, an imported species to Iran, successfully compatible with green space of different regions of Iran

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together with *A. millefolium* (white flower), were reported as a superior species.

Phyla nodiflora L. (*Lippia nodiflora* (L.) Mihex) is a member of the family Verbenaceae, distributed in India, Ceylon, Baluchistan, South Africa and Central America (Terblanche and Kornelin, 1996). *Lippia* is a prostrate perennial broadleaf herb and has many branched stems, sometimes up to a meter in length. The plant has the ability to send down roots at nodes along the stems, and when well established *Lippia* can form a low maintenance and dense mat of ground cover species. *Lippia* was introduced in some areas as a soil stabilizer. Because of its massive tap root system, *Lippia* has the ability to draw moisture from very deep levels in the soil profile. Instead of stream banks being stabilized, they slump and collapse as a result of the soil being dried out to several meters. Its leaves are eaten in Ceylon and taken as tea in the Philippines. It is an aromatic, runner-producing plant with scanty roots and is used as a cure for adenopathy, chronic indolent ulcers, diuretic and aphrodisiac and is also used for treatment of heart diseases, ulcers, bronchitis, fevers, and colds (Kirtikar and Basu, 1975). Ravikanth et al. (2000) reported the anticancer compounds (halleridone and hallerone) from *P. nodiflora*.

White clover (*Trifolium repens* L.) is an herbaceous perennial legume that reproduces from seed as well as vegetatively from stolons. Under hot and dry conditions this plant die back to the stolons, but with prolonged summer drought the stolons would be died as well. In autumn, it will show a second smaller flush of vegetative growth. They will go to a slight dormancy in winter especially where cold conditions prevail. White clover is winter hardy and frost tolerant but has poor growth when the soil temperature is <5°C.

Frankenia thymifolia Desf is an evergreen, downy and interlace groundcover (Ghahreman, 2000). It belongs to Frankeniaceae family which is considerably saline and drought tolerant and suitable for saline soil reclamation (Easton and Kleindorofer, 2009). In a comparative study between sport turfgarass and *F. thymifolia* L., the annual maintenance costs of *F. thymifolia* in 100 m^2 was almost twice less than sport lawns and it required approximately 80% less water compared to sport lawn which is a mixture of *Cynodon dactylon* L. [Pers.], *Poa pratensis* L. and *Festuca rubra* L. (Shooshtarian and Tehranifar, 2010).

Periwinkle (*Vinca minor* L.) is an angiosperm and a cold tolerant, herbaceous evergreen plant commonly used as a ground cover. The leaves of this species that develop in the spring and summer are the same leaves that subsequently are exposed to low temperature and freezing conditions of autumn and winter (Huner et al., 1988). Shooshtarian and Salehi (2011) studied 10 species of groundcover plants in 4 regions of Kish landscape and reported that *Glaucium flavum* Crantz. *Achillea milleifolium* L. and *Carpobrotus acinaciformis* (L.) L. Bolus., are proper groundcover plants for Kish green spaces.

Acar and Var (2001) studied compatibility and ornamental potential of 19 groundcover plant species, endemic in Trabazon province (Turkey). They recommended two species of *Sedum spurium* and *Thymus praecox* for application in the urban green space because of their acclimation and high levels of coverage. Dou et al. (2004) recommended three out of 205 species of groundcover plants native to Yunnan province (China). Those three species were recommended due to their growth habit and ornamental features for urban landscape of tropical regions.

Responses of five Mediterranean halophytes (*Sarcocornia fruticosa* (L.) A.J. Scott, *Inula crithmoides* L., *Plantago crassifolia* Forssk., *Juncus maritimus* Lam. and *J. acutus* J. Brun.) to seasonal changes in environmental conditions were studied by Gil et al. (2014). They proposed that tolerance mechanisms based on the control of ion transport and osmolyte biosynthesis, are efficient enough to avoid excessive ROS production and oxidative damage in the studied plants.

The amino acid proline normally accumulates in large quantities in higher plants in response to environmental stresses (Khedr et al., 2003). Plants employ antioxidant defense mechanisms against oxidative damage of reactive oxygen species. Some of the proteins made in response to such stresses are enzymes that synthesize compounds believed to serve as osmoprotectants, including proline, polyamines and glycine betaine (Udomchalothorn et al., 2009).

There are just few reports on establishment, growth, physiological and biochemical characteristics of ground covers during different months of the growing season in the natural environmental conditions. The aim of the present study was to investigate the seasonal changes in physiological and biochemical characteristics of the above mentioned plants in Shiraz city, Iran.

Materials and Methods

Plant materials and experimental conditions

This research was conducted at Eram Botanical Garden located in Shiraz (29^{o} 38' N and 52^{o} 31' E, 1480 m). Some meteorological parameters of the experimental region during study are shown in Table 1.

Table 1. Meteorological data of experimental region (2013).

Month	Average monthly temperature (°C)			Sunny hours	Evaporation (mm)	Precipitation (mm)	Average relative humidity (%)		
	Mean	Max	Min				Mean	Max	Min
Apr.	15.6	23.2	8	273.1	151.7	99.9	50	76	24
May.	18.3	25.9	10.7	301.1	224.1	41.9	44	69	20
Jun.	26.1	35.5	16.7	370	371.4	0	24	41	7
Jul.	29.5	39.2	19.8	366.1	430.6	0	25	43	7
Aug.	29.9	38.6	21.2	337.8	387	3.5	29	47	11
Sep.	26.1	35.7	16.6	320.1	309.7	0	28	48	7
Oct.	20.3	30.5	10.1	310.3	216.5	0	32	54	10
Nov.	14.2	21.1	7.4	215.3	94.6	101.2	58	84	32
Dec.	9.8	17.2	2.3	245.7	58.9	1.1	55	83	27
Jan.	3.2	8.7	-2.2	169.6	0	94.7	66	87	46
Feb.	6.9	14.2	-0.3	249.1	0	92.2	56	85	27

Source: Meteorological Station of Shiraz, Iran

Plant materials were collected from Eram Botanical Garden nurseries and green spaces of Agricultural College of Shiraz University. Six ground cover plants that were used in the current study consisted of: *Vinca minor* L. 'Variegatum', *Vinca minor* L. 'Green', *Oxalis brasiliensis* G. Lodd, *Trifolium repens* L., *Phyla nodiflora* L. and *Frankenia thymifolia* Desf. The plants were cultured 25×25 cm^2 apart in plots (1 × 3 m^2) that were previously prepared by plowing and leveling the soil. Irrigation interval was one week according to its local cultivation method. Soil test was done before planting. The soil texture was sandy loam, pH was 7.8 and EC of the soil extract was 0.77 dsm^{-1}. Total nitrogen, potassium and phosphor contents were 0.57%, 530 mg Kg^{-1} and 18 mg Kg^{-1}, respectively. Plants were cultured in mid-April 2013. Samples were collected every two months (mid-month) after the culturing time.

• *Chlorophyll content and Relative Water Content (RWC)*

RWC calculation was performed by incubating 0.2 g of fresh leaf sample in 50 ml of distilled water for 4 h. Then, the turgid weights of leaf samples were measured. The leaf samples were oven dried for calculating dry weight at 70 °C for 48 h. The RWC was determined by the following equation:

$$\mathrm{RWC}(\%) = (f.w - d.w / t.w - d.w) \times 100$$

where: f.w. is fresh-, d.w. is dry- and t.w. is turgid-weight (Nepomuceno et al., 1998; Sairam et al., 2002).

Chlorophyll content was estimated by the method of Saini et al. (2001) using the following formula:

$$\text{mg Chl / g f.w.} = [(20.2(OD645nm) + (8.02(OD663\ nm)] \times v / f.w \times 1000$$

Where: OD is optical density, v is the final solution volume in ml and f.w. is tissue fresh weight in mg.

- ***Electrolyte Leakage (EL) and Proline content***

EL in leaves measured, as described by Saadalla et al. (1990) using an electrical conductivity meter (Metrohm 644, Swiss) and calculated with the following formula:

$$\text{EL}(\%) = \left(\frac{EL1}{EL2}\right) \times 100$$

Proline content was determined according to the method described by Bates et al. (1973). Using a spectrophotometer (UV-120-20, Japan) at 520 nm wavelength, appropriate proline standards were included for calculation of its concentrations in the samples.

- ***Extraction and assay of antioxidant enzymes***

Frozen leaf segments (0.5 g) were ground with liquid nitrogen and 2 ml of extraction buffer containing Tris (6.07 g) and PVP (0.5 g) (pH 8.0) was added in ice bath. The homogenate was centrifuged at 13000 × g for 20 min at 4 °C. Supernatant was used for enzyme activity assay. All steps in the preparation of the enzyme extract were carried out at 4°C. All spectrophotometric analyses were conducted on a Shimadzu (UV-2401PC spectrophotometer.

Superoxide dismutase (SOD) activity was determined based on the method described by Beauchamp and Fridovich (1971), which measures the inhibition in the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction which was measured at 560 nm.

Guaiacol peroxidase (GPX) activity was measured using modification of the procedure of Chance and Maehly (1995), the reaction mixture contained 50 mM (pH 7.0) potassium phosphate buffer, 13 mmol guaiacol, 5 mM H_2O_2 and 33µl enzyme extract. The increase of absorbance due to oxidation of guaiacol was measured at 470 nm (E = 26.6 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981), the reaction mixture consisted of 50 mM (pH 7.0) potassium phosphate buffer, 0.15 mM EDTA, 0.5 mM ascorbate, 0.15 mM H_2O_2 and 50 µl enzymes extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (E = 2.8 mM^{-1} cm^{-1}).

Catalase (CAT) activity was calculated according to Chance and Maehly (1995) method. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2. The reaction was initiated by addition of 50 µl of enzyme extract and activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm (E = 39.4 mM^{-1} cm^{-1}) for 60 s.

- ***Total soluble sugars and starch analysis***

Phenol–sulfuric acid method was used to determine the total soluble sugars content (Dubois et al., 1956; Buysse and Merckx, 1993). Starch content in shoots was measured with the method described by McCready et al. (1950).

- ***Statistical analysis***

Statistical analysis was done with SAS (ver. 9.1.3) software. Experiment was conducted in a complete randomized blocks design with three replications and means were compared using the least significant difference (LSD) test at $p < 0.05$.

Results

As shown in Table 2, the results of analysis of variance for most biochemical characteristics of *V. minor* ‘Variegatum’ L are significant at 5% level. Chlorophyll content and RWC had a decreasing trend from June to August. During the autumn, these characteristics were increased. Electrolyte leakage was decreased in August while it was increased in February. Proline content was significantly increased in February compared to its content in other months. The total soluble sugar content increased from June to December. The starch content decreased until mid-summer while it accumulated in autumn and winter. SOD, APX and CAT activity increased from June to August but APX activity decreased during the same period. However, except for CAT the activity of antioxidant enzymes were significantly increased during winter (Table 3).

Table 2. Analysis of variance for physiological and biochemical traits of *Vinca minor* L.‘Variegatum’ under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	0.182 ns	207.836*	286.662**	0.018*	4770.293**	17037.002**	0.692**	423.365**	4156.400**	33.974*
Replication	2	0.065	183.824	44.834	0.003	442.11	50.952	0.002	0.07	56.267	4.082
Error	8	0.127	41.341	33.392	0.003	133.553	111.54	0.01	16.443	195.6	5.134
CV(%)		15.637	8.579	23.198	24.384	8.691	9.307	22.985	11.301	14.711	22.591

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 3. Seasonal changes in some parameters of *Vinca minor* L.‘Variegatum’.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	4.9±0.13	3.901±0.11	5.27±0.12	5.27±0.12	7.33±0.37
Relative water content (%)	68.68±2.64	66.77±2.25	71.86±5.19	85.70±7.21	81.71±5.04
Electrolyte leakage (%)	25.09±6.38	11.44±1.75	28.71±3.06	21.26±2.17	38.06±1.25
Proline (µmol g^{-1} f.w.)	0.14±0.01	0.23±0.025	0.20±0.01	0.23±0.05	0.36±0.05
Total soluble sugar (mg g^{-1} d.w.)	102.64±2.90	101.56±6.11	165.43±8.06	186.20±11.90	109.06±8.55
Starch content (mg g^{-1} d.w.)	65.67±3.79	39.89±4.67	72.67±8.60	184.68±7.27	204.47±1.65
SOD activity	88.67±4.06	101.33±5.70	36.67±4.06	111.33±5.93	137.30±13.40
POD activity	0.07±0.01	0.08±0.01	0.50±0.06	0.27±0.03	1.23±0.09
APX activity	25.36±1.22	20.71±1.40	42.92±3.93	45.41±1.25	45.01±1.21
CAT activity	12.24±0.89	13.22±2.48	11.42±0.78	8.26±0.45	5.01±0.65

Data are presented as means ± standard error.

The analysis of variance in Table 4 revealed significant difference in chlorophyll content, total soluble sugars, and POD, SOD and CAT activities.

In *V. minor*, chlorophyll content, RWC, EL, proline and total soluble sugars, antioxidant enzymes activities were decreased from June to August. However, SOD and POD enzymes were significantly increased in autumn and winter. From mid-spring to mid-summer, CAT activity increased and then decreased throughout autumn and winter. The starch concentration rose continuously until the mid-autumn, and then reduced during winter (Table 5).

Table 4. Analysis of variance for physiological and biochemical traits of *Vinca minor* L. under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	3587.128*	95.407ns	43.562ns	0.010ns	13.502*	2.064ns	0.653**	1.009ns	4037.600**	3.021**
Replication	2	15.778	129.647	4.147	0.004	1.125	15.903	0.019	3.06	1049.867	0.008
Error	8	21.115	43.888	15.026	0.004	2.846	11.192	0.009	1.196	263.2	0.399
CV(%)		20.499	8.134	28.821	15.343	14.747	27.381	14.822	22.186	15.926	20.12

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 5. Seasonal changes in some parameters of *Vinca minor* L.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	5.40±0.15	4.75±0.62	10.46±0.69	7.32±1.42	7.57±0.78
Relative water content (%)	76.24±4.27	75.67±1.10	82.04±3.73	89.13±6.15	84.15±5.53
Electrolyte leakage (%)	12.47±0.79	7.59±1.45	13.53±1.76	16.75±3.45	16.91±1.92
Proline (µmol g^{-1} f.w.)	0.47±0.07	0.37±0.03	0.42±0.01	0.46±0.02	0.34±0.01
Total soluble sugar (mg g^{-1} d.w.)	11.21±1.67	8.86±06	13.30±0.95	13.84±0.072	9.99±0.24
Starch content (mg g^{-1} d.w.)	11.25±2.33	11.46±3.36	12.67±1.42	13.20±0.49	12.50±1.13
SOD activity	76.70±12.80	72.00±6.93	88.67±7.69	110.00±4.62	162.00±20.2
POD activity	0.63±0.05	0.26±0.04	0.45±0.04	0.41±0.03	1.44±0.11
APX activity	4.01±0.351	4.91±0.14	4.91±1.02	5.57±0.34	5.24±1.15
CAT activity	16.24±2.43	16.01±2.95	5.09±0.89	3.99±1.89	13.01±1.2

Data are presented as mean ± standard error

Table 6. Analysis of variance for physiological and biochemical traits of *Trifolium repens* L. under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	35.235**	48.549ns	0.610*	0.981**	3471.734*	11.219ns	1.846*	0.030ns	23.182ns	1.164*
Replication	2	0.18	318.06	1.638	0.015	656.938	11.385	0.125	0.061	15.157	0.925
Error	8	2.724	96.303	0.117	0.065	542.978	4.489	0.464	0.089	20.723	0.261
CV(%)		17.872	11.75	9.73	21.219	23.992	23.666	24.326	26.41	28.74	18.371

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 7. Seasonal changes in some biochemical and physiological parameters in *Trifolium repens* L.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	5.963±0.55	5.18±1.31	11.06±0.33	12.81±0.21	11.16±1.23
Relative water content (%)	83.44±5.52	79.76±4.22	79.90±2.33	89.46±5.95	85±12.10
Electrolyte leakage (%)	16.14±0.36	16.14±0.36	9.90±0.35	9.58±0.27	12.21±0.50
Proline (µmol g^{-1} f.w.)	0.48±0.14	3.49±0.17	0.64±0.00	3.21±0.14	0.34±0.15
Total soluble sugar (mg g^{-1} d.w.)	65.32±3.27	79.89±6.83	105.0±20.7	82.99±9.73	152.4±19.1
Starch content (mg g^{-1} d.w.)	44.07±0.01	84.2±2.67	143.04±0.14	73.66±0.26	90.3±1.60
SOD activity	311.3±1.43	252.0±1.10	417.0±4.28	171.3±2.47	199.3±2.23
POD activity	2.97±0.28	1.69±0.55	2.36±0.48	3.29±0.16	3.68±0.11
APX activity	1.11±0.48	1.13±0.64	1.15±0.89	0.99±0.47	1.29±0.97
CAT activity	11.35±0.33	10.98±0.04	4.84±0.037	9.91±0.6	4.52±0.43

Data are presented as mean ± standard error.

In *T. repens*, according to the result obtained from the analysis of variance significant differences at 5% level were observed for chlorophyll content, proline, EL, total soluble sugars, POD and CAT activities under seasonal changes (Table 6).

Leaf chlorophyll content and RWC were decreased from June to August in leaves of *T. repens*, while its maximum value was observed in December. Seasonal changes in EL and proline content were not significant in *T. repens*. In addition, total soluble sugars and starch contents were increased from June to October, then slightly decreased in December and again raised in February. SOD and POD activities were decreased from June to August and then increased in December and increased again in February (Table 7).

Statistical analyses for the evaluated traits in *P. nodiflora* L. were shown in Table 8. There were significant differences in RWC, El, total soluble sugars and POD at 5% level due to seasonal changes. Chlorophyll content was slightly decreased in the mid-summer and was significantly decreased in winter. In addition, EL and proline content were the lowest in October compared to other months. Total soluble sugars were continuously increased from June to December, while their contents were decreased in February. Starch accumulation occurred in December and decreased in February. Antioxidant enzymes showed significant differences among different months. SOD and CAT activities were notably decreased from mid-June to mid-December. However, POD and APX activities significantly decreased in mid-summer and rose in mid- winter (Table 9).

In *F. thymifolia*, as indicated in Table 10, there were significant differences in chlorophyll content, RWC, proline, total soluble sugars, and POD, SOD and CAT activities. RWC was significantly decreased in December compared to other months, while no significant difference was found in regard to chlorophyll content among other months. Highest EL and proline content were observed in mid-autumn and mid-winter, respectively. Total soluble sugars were continuously increased from mid-June to mid-February. In addition, starch content was increased from mid-June to mid-October but slightly reduced in December and February. SOD and POD activities were continuously increased from June to February. CAT and APX activities also were increased in February (Table 11).

Table 8. Analysis of variance for physiological and biochemical traits of *Phyla nodiflora* L. under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	2.368ns	143.613*	20.890**	0.345	49.929**	23.484ns	0.731**	1.607ns	49.497ns	0.767ns
Replication	2	0.406	97.859	41.83	0.013	2.832	3.688	0.021	0.698	2.029	0.165
Error	8	0.705	25.592	2.994	0.028	3.066	7.946	0.055	0.757	19.188	0.232
CV(%)		17.726	6.021	14.055	20.254	15.846	21.927	22.439	21.711	25.222	19.172

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 9. Seasonal changes in some physiological and biochemical traits of *Phyla nodiflora* L.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	5.14±0.28	4.75±0.77	5.72±0.41	4.75±0.42	3.31±0.22
Relative water content (%)	81.36±4.17	80.69±2.17	80.00±2.39	96.35±3.65	81.73±5.06
Electrolyte leakage (%)	14.24±1.87	14.24±1.87	12.04±2.15	7.88±0.34	13.14±2.48
Proline (µmol g^{-1} f.w.)	0.49±0.06	0.23±0.09	1.39±0.15	0.37±0.08	1.47±0.03
Total soluble sugar (mg g^{-1} d.w.)	28.83±0.24	88.00±0.25	218.4±2.12	239.04±0.13	112.8±0.63
Starch content (mg g^{-1} d.w.)	175.2±2.22	130.3±1.47	231.5±1.92	260.2±0.89	84.19±0.52
SOD activity	193.3±0.27	184.0±1.89	475±4.03	270.00±0.03	504.0±2.52
POD activity	1.33±0.07	0.063±0.03	1.16±0.18	2.59±0.11	1.41±0.17
APX activity	16.45±0.34	8.47±0.44	21.71±0.29	15.30±0.19	22.94±0.89
CAT activity	10.12±0.09	7.26±0.37	4.15±0.19	4.03±0.16	7.78±0.39

Data are presented as mean ± standard error.

Table 10. Analysis of variance for physiological and biochemical traits of *Frankenia thymifolia* Desf. under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	0.559**	322.361*	160.888ns	1.676*	50.746**	0.482ns	0.104**	0.529ns	51.583**	0.923**
Replication	2	0.135	22.64	20.171	0.349	0.19	0.145	0.011	0.06	17.635	1.091
Error	8	0.044	49.818	50.485	0.404	2.57	0.143	0.005	0.287	4.976	0.124
CV(%)		8.449	9.716	13.182	18.772	17.523	20.7	19.948	13.619	19.944	15.083

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 11. Seasonal changes in some physiological and biochemical traits of *Frankenia thymifolia* Desf.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	4.5±0.01	4.03±0.14	8.02±0.11	9.07±0.26	6.41±0.08
Relative water content (%)	69.92±2.91	69.89±1.81	76.01±4.53	59.55±3.42	87.87±5.48
Electrolyte leakage (%)	55.46±4.92	55.46±4.92	64.32±2.57	49.30±3.82	44.96±2.11
Proline (µmol g^{-1} f.w.)	12.67±0.17	13.89±0.21	7.72±0.15	7.16±0.63	19.44±0.40
Total soluble sugars (mg g^{-1} d.w.)	19.71±0.76	30.16±0.19	105±1.07	191.6±0.81	146.6±1.03
Starch content (mg g^{-1} d.w.)	28.57±0.18	60.1±0.27	294.6±0.01	111.3±0.30	72.5±0.21
SOD activity	46.67±0.52	78.7±1.74	128.7±0.18	134.7±0.37	331±3.01
POD activity	0.03±0.04	0.04±0.03	0.11±0.03	0.34±0.07	0.29±0.05
APX activity	17.04±0.25	14.38±0.18	12.96±0.19	13.13±0.31	21.34±0.42
CAT activity	6.89±0.47	9.04±0.36	2.54±0.29	4.13±0.06	6.90±0.30

Data are presented as mean ± standard error.

For *Oxalis brasiliensis*, ANOVA analysis showed significant differences at 5% level in chlorophyll content, RWC, total soluble sugars, starch content, POD and SOD activities among different seasons. The results indicated that chlorophyll content was significantly increased in autumn and winter. There was no significant difference in EL among other months. However, highest EL was detected in mid-winter. Proline content was significantly decreased in October. Total soluble sugars were significantly increased from June to February. Highest starch content was observed in October and a slight decrease was found in December and February. SOD and POD activities were maximum during late autumn and mid-winter. APX and CAT activities were at the lowest levels in October and February, respectively (Table 13).

Table 12. Analysis of variance for physiological and biochemical traits of *Oxalis brasiliensis* G. Lodd.. under seasonal changes

Source of variation	DF	Chl	RWC	EL	Proline	TSS	ST	POD	APX	SOD	CAT
Treatment	4	8200.548**	7.630*	20.682ns	0.382ns	81.474**	0.098*	0.399**	1.307ns	17.948*	0.036ns
Replication	2	545.846	9.008	0.089	0.787	18.102	0.009	0.009	0.472	0.671	0.003
Error	8	343.143	1.744	0.542	0.357	19.108	0.026	0.007	0.600	2.957	0.023
CV(%)		17.346	18.263	17.815	18.603	5.274	38.449	21.531	18.741	15.178	32.382

** and *Significantly different at 1% and 5% respectively.
ns: Non significant

Table 13. Seasonal changes in some physiological and biochemical traits of *Oxalis brasiliensis* G. Lodd.

Parameters	June	August	October	December	February
Chlorophyll content (mg g^{-1} f.w.)	1.84±0.09	1.20±0.16	4.16±0.40	6.55±0.59	6.93±0.46
Relative water content (%)	82.06±1.6	75.74±0.98	89.34±4.3	86.30±1.43	80.98±2.73
Electrolyte leakage (%)	9.13±0.23	9.13±0.23	10.10±0.31	9.75±0.41	15.50±0.60
Proline (µmol g^{-1} f.w.)	0.25±0.05	0.36±0.16	0.12±0.02	0.18±0.02	0.29±0.09
Total soluble sugars (mg g^{-1} d.w.)	50.92±1.18	50.72±5.76	144.9±22.70	128.07±8.32	159.36±4.37
Starch content (mg g^{-1} d.w.)	32.47±0.29	47.69±0.75	46.7±1.21	101.9±1.31	52.3±1.23
SOD activity	82.67±0.31	117.3±1.15	82.0±0.32	198±1.53	194±0.54
POD activity	0.02±0.01	0.04±0.01	0.02±0.01	0.94±0.06	0.29±0.09
APX activity	19.61±0.26	19.81±0.25	8.81±0.09	21.75±0.68	19.10±0.60
CAT activity	15.01±0.17	15.18±0.79	7.911±0.30	10.46±0.28	4.298±0.10

Data are presented as mean ± standard error.

Discussion

Results obtained from present study suggest that *P. nodiflora* L. and *T. repens* were vigorously spread during the first month and the whole area was covered in comparison with other ground cover plants. This is due to the stoloniferous growth habit of these plants which prevented weed infestation. The only problem of *P. nodiflora* L. is yellowing and browning of upper branches with low temperatures and short day-length during autumn and winter seasons. It appears that with fertilizer management this problem can be properly removed. *T. repens* L. and *V. minor* maintained their performance during experiment. Ranking of the studied plants based on their establishment rate after the first month can be as: *P. nodiflora*, *T. repens*, *F. thymifolia*, *V. minor*, *V. minor* 'Variegatum' L. and *O. brasiliensis*, respectively.

Color change of leaves in *P. nodiflora* and *F. thymifolia* was increased during autumn and winter. *P. nodiflora* had pale green and brown colors in upper leaves, during autumn and winter. *F. thymifolia* had a purple color in shoot upper parts in autumn and winter. Therefore, *P. nodiflora* can be used as a grass substitute for lawns in tropical areas. It is also evergreen in areas protected from frost; and generally, is a good nectar producing plant for butterflies. It is an attractive plant rambling over boulders or the edges of hanging baskets. It also can tolerate drought and flooding. Results showed that leaf chlorophyll content of *P. nodiflora* was more affected by low temperatures. Decrease in chlorophyll content due to low temperature has been also reported in *Poa pratensis* L., *Roegneria thoroldiana* and *Elymus nutans*, the major reason was decreased stability of the chloroplast during stress and disturbance in the chlorophyll later (Yan et al., 2007).

There are not so many reports on the effect of low temperature upon seasonal changes on chlorophyll content of these ground cover plants. At cold months (15-20 ^{o}C), there was also an increase in the chlorophyll content in two species of *Vinca*, *T. repens*, *O. brasiliensis* and *F. thymifolia*.

In the current study, the highest proline content was found in *F. thymifolia* Desf. in mid-summer and mid-winter, while other ground cover plants were not shown significant differences for their proline contents.

The EL method is a god estimate for predictive capability of low temperature survival. Among different months, EL of *F. thymifolia* leaves was lowest in August.

The most damage to the cell membrane of *Vinca minor* ʽVariegatumʼ L. was observed in February. In addition, *T. repens* L. was exhibited the highest EL in October. Therefore, it appears that *F. thymifolia* is more tolerant to higher temperatures compared to low and freezing temperatures during experiment. While, *Vinca minor* ʽVariegatumʼ L. and *T. repens* L. showed more sensitivity with low temperatures in autumn and winter.

Antioxidant enzymes showed various patterns during seasonal changes. No difference in SOD and GPX activities were found from June to August. However, SOD activity in *T. repens* and *P. nodiflora* were more than that in other plants.

GPX activity of *T. repens* increased from August to February. CAT activity was fluctuated in response to seasonal changes in ground cover plants. It appears that antioxidant enzyme activity was more affected during autumn and winter times compared to warm seasons. CAT Activity was notably decreased in all studied plants in October, while it was increased in *Frankenia*, *Oxalis* and *Phyla*.

Temperature plays a key role in the accumulation and mobilization of carbohydrates in plants. Soluble sugar levels were notably increased in *Phyla* from June to December and then were decrease in February, while starch levels were decreased from June to August. Evidence suggests that soluble sugars, such as sucrose and oligosaccharides of the raffinose family, in combination with heat stable proteins play a determinant role in stress tolerance by protecting proteins and membranes against freezing-induced denaturation (Gusta et al., 1996).

In all studied plants, soluble sugar levels were enhanced by increasing temperature. Reducing sugar and starch levels of *T. repens* leaves were decreased in mid-autumn.

During the autumn, as plant growth slows in response to low temperatures and short days (Boller and Nosberger, 1983), carbon (C) is not utilized and thereby accumulates in carbohydrate form in the stolons and roots of *T. repens* L. (Baur-Höch, Machler and Nosberger, 1990). Approximately 10% of assimilated C is invested in long-term storage in roots and stolons. These C compounds serve as a C source during periods when respiration exceeds photosynthesis (Danckwerts and Gordon, 1989). Böller and Nösberger (1983) found that although the stolons contain the highest amount of accumulated non-structural carbohydrates, the relative increase in non-structural carbohydrate content of the roots is highest when temperature and photoperiod are decreased. Therefore, it seems that the roots are an important C sink in *T. repens* L. in autumn and winter. Frankow -Lindberg (2001) found that white clover plants adapted to an environment with long overwintering periods have slower rates of C reserve utilization at low positive temperatures compared with plants of a more southern origin. They stated that the cultivars that adapted to sub-lethal frosts were able to conserve total non-structural carbohydrate (TNC) concentration in both stolons and roots when exposed to frequent sub-lethal frosts. On the other hand TNC concentration does not preserve in those parts of non-adapted cultivars. Similar studies were conducted on bermudagrass (Macolino et al. 2010; Schiavon et al., 2016). They concluded that there was positive correlation between water-soluble carbohydrates and winter survival of bermudagrass cultivars.

In conclusion, *T. repens*, *V. minor*, *F. thymifolia* and *P. nodiflora* can be recommended for cultivation in Shiraz green spaces and the same conditions elsewhere. *V. minor* ‘Variegatum’ was very sensitive to seasonal changes.

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References

1. Acar C, Var M. 2001. A study on the adaptations of some natural ground cover plants and on their implications in landscape architecture in the ecological conditions of trabzon. Turkish Journal of Agriculture and Forestry 25, 235-245.
2. Ashraf M, Foolad M.R. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59, 206-216.
3. Baur-Höch B, Mächler F, Nösberger J. 1990. Effect of carbohydrate demand on the remobilization of starch in stolons and roots of white clover (*Trifolium repens* L.) after defoliation. Journal of Experimental Botany 41, 573-578.
4. Beauchamp C, Fridovich I. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44, 276-287.
5. Britz S, Hungerford J, Lee D.R. 1985. Photoperiodic regulation of photosynthate partitioning leave of *Digitaria dacumbens* Stent. Plant Physio l78, 710-4.
6. Buysse J, Merckx R. 1993. An improved colorimetric method to quantify sugar content of plant tissue. Journal of Experimental Botany 44, 1627-1629.
7. Böller B, Nösberger J. 1983. Effects of temperature and photoperiod on stolon characteristics, dry matter partitioning, and nonstructural carbohydrate concentration of two white clover ecotypes. Crop Science 23, 1057-1062.
8. Chance B, Maehly A.C. 1955. Assay of catalase and peroxidases. Methods in Enzymology. 2, 764-765.
9. Danckwerts J.E, Gordon A.J. 1989. Long-term partitioning, storage and remobilization of ^{14}C assimilated by *Trifolium repens* (cv. Blanca). Annals of Botany 64, 533-544.
10. Dou J, Zhou S.U, Xu Z.F. 2004. Tropical native ground cover plants resources of southern Yunnan and their sustainable use in landscape. Journal of Zhejiang Forestry College 1, 54-61.
11. Dubois M, Gilles K.A, Hamilton J.K, Rebers, P.A, Smith F. 1956. Colorimetric method for determination of sugar and related substances. Analytical Chemistry 28, 350–356.
12. Easton C.L, Kleindorfer S. 2009. Effects of salinity levels and seed mass on germination in Australian species of *Frankenia* L. (Frankeniaceae). Environmental and Experimental Botany 65, 345-352.
13. Frankow -Lindberg B.E. 2001. Adaptation to winter stress in nine white clover populations: changes in non-structural carbo-hydrates during exposure to simulated winter conditions and ‘Spring’ regrowth potential. Annals of Botany 88, 745-751.
14. Ghahreman A. 2000. Final report of plant cover study program and floristic of Kish Island. Kish Island, pp. 205.
15. Ghani A, Azizi M, Tehranifar A. 2010. Potential evaluating of five ornamental species of wild *Achillea* cultivated under Mashhad climate. Iran Horticultural Science Journal (Agriculture Science and Industry) 32, 25-31.
16. Gil R, Bautista I, Boscaiu M, Lidón A, Wankhade S, Sánchez H, Llinares J, Vicente O.

2014. Responses of five Mediterranean halophytes to seasonal changes in environmental conditions. AoB Plants 6.

17. Gusta L.V, Wilen R.W, Fu P. 1996. Low-temperature stress tolerance: The role of abscisic acid, sugars, and heat-stable proteins. HortScience 31, 39-46.

18. Hoekstra F.A, Golovina E.A, Buitink J. 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science 6, 431-438.

19. Huner N.P.A, Krol M, Williams J.P, Maissan E. 1988. Overwintering periwinkle (*Vinca minor* L.) exhibits increased. Plant Physiology 87, 721-726.

20. Khedr A.A, Abbas M.A, Abdel Wahid A.A, Quick W.P, Abogadallah G.M. 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. Journal of Experimental Botany 45, 2553-2562.

21. Kirtikar K.R, Basu B.D. 1975. In: Indian medicinal plants, 2nd ed. Jayeed Press, New Delhi 3, 1916 -1917.

22. Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiology and Biochemistry 45, 244-249.

23. Mächler F, Nösberger J. 1977. Effect of light intensity and temperature on apparent photosynthesis of altitudinal ecotypes of *Trifolium repens* L. Oecologia 31, 73-78.

24. Macolino S, Serena M, Leinauer B, Ziliotto U. 2010. Preliminary findings on the correlation between water soluble carbohydrate content in stolons and first year green-up of seeded bermudagrass cultivars. HortTechnology 20, 758-776.

25. McCready R.M, Guggolz J, Silviera V, Owens H.S. 1950. Determination of starch and amylase in vegetables. Analytical Chemistry 22, 1156–1158.

26. Montague T, Kjelgren R.K, Rupp L. 2000. Surface energy balance affects gas exchange and growth of two irrigated landscape tree species in an arid climate. Journal of the American Society for Horticultural Science 125, 299-309.

27. Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant and Cell Physiology 22, 867-880.

28. Naz S, Ahmad S, Rasool S.A, Sayeed. S.A, Siddiqi R. 2006. Antibacterial activity directed isolation of compounds from *Onosma hispidium*. Microbial Resolution 161, 43-48.

29. Pittenger D.R, Shaw D.A, Hode D.R, Holt D.B. 2001. Responses of landscape ground covers to minimum irrigation. Journal of Environmental Horticulture 19, 78-84.

30. Ravikanth V, Ramesh P, Diwan P.V, Venkateswarlu Y. 2000. Halleridone and Hallerone from *Phyla nodiflora* (L.) Greene. Biochemical Systematic Ecology 28, 905-906.

31. Saadalla M.M, Shanahan J.F, Quick J.S. 1990. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. Crop Science 30, 1243-1247.

32. Saini R.S, Sharme K.D, Dhankhar O.P, Kaushik R.A. 2001. Laboratory manual of analytical techniques in horticulture. India: Agrobios 49-50.

33. Sairam R.K, Rao K.V, Srivastava G.C. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science 163, 1037-1046.

34. Samieiani E, Ansari H. 2014. Drought Stress Impact on Some Biochemical and Physiological Traits of 4 Ground covers (*Lolium perenne, Potentilla spp*, *Trifolium repens* and *Frankinia spp*) with Potential Landscape Usage. Journal of Periodontology 4, 53-60.

35. Schiavon M, Macolino S, Leinauer B, Ziliotto U. 2016. Seasonal changes in carbohydrate and protein content of seeded bermudagrasses and their effect on spring green-up. Journal of Agronomy and Crop Science 202, 151-160.

36. Shooshtarian S, Salehi H. 2011. Physiological and ecological investigation on adaption of some ground cover plants as turfgrass alternatives in arid landscape region of Kish Island during cool season. African Journal of Agricultural Research 7, 546-554.

37. Shooshtarian S, Tehranifar A. 2010. The study of application of xerophile ground cover plants in urban landscape of Mashhad. Journal of Mashhad Pazhoohi 1, 92-105.

38. Terblanche F.C, Kornelius G. 1996. Essential oil constituents of the genus *Lippia* (Verbenaceae) – A literature review. Journal of Essential Oil Research 8, 471-485.

39. Udomchalothorn T, Maneeprasobsuk S, Bangyeekhun E, Boon-Long P, Chadchawan S. 2009. The role of the bifunctional enzyme, fructose-6-phosphate-2-kinase/fructose-2, 6-bisphosphatase, in carbon partitioning during

salt stress and salt tolerance in Rice (*Oryza sativa* L.). Plant Science 176, 334-341.

40. Van der Spuy U. 1976. Gardening with groundcovers. A.H. and A.W. Reed Wellington. pp. 112.
41. Vranova E, Inzé D, Van Breusegem F. 2002. Signal transduction during oxidative stress. Journal of Experimental Botany 53, 1227-1236.
42. Wachendorf M, Collins R.P, Elgersma A, Fothergill M, Frankow-Lindberg B.E, Ghesquiere A, Guckert A, Guinchard M.P, Helgadottir A, Lüscher A, Nolan T, Nykänen-Kurki P, Nösberger J, Parente G, Puzio S, Rhodes I, Robin C, Ryan A, Stäheli B, Stoffel S, Taube F, Connolly J. 2001. Overwintering and growing season dynamics of *Trifolium repens* L. in mixture with *Lolium perenne* L.: a model approach to plant–environment interactions. Annals of Botany 88, 683-702.
43. Weston L.A. 2002. Weed suppressive ground covers, a more attractive and effective way to manage weeds. Cornell University Turfgrass Times (CUTT) 12, 4-7.
44. Yang S.Q, Ren G.X, Yang G.H, Feng Y.Z, Zhang Q. 2007. Effects of Water stress on osmoregulation substances and chlorophyll fluorescent parameter for forage grass. Acta Botanica Boreal-occident Sinicia 27, 1826-1832.
45. Yang W.L, Liu J.M, Chen F, Liu Q, Gong Y.D, Zhao N.M. 2006. Identification of *Festuca arundinacea* Schreb. Cat1 Catalase Gene and analysis of its expression under abiotic stresses. Journal of Integrative Plant Biology 48, 334-340.