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Drought Stress Impact on Some Biochemical and Physiological Traits of 4 Groundcovers (*Lolium perenne*, *Potentilla* spp, *Trifolium repens* and *Frankinia* spp) with Potential Landscape Usage

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Abstract

Stress management is considered as an important factor in nowadays landscape. Therefore this research was conducted using a factorial experiment based on a randomized completely design with regulated deficit irrigation at four levels (100, 75, 50, and 25% of lawn irrigation requirement) and three replicates to evaluate some biochemical and physiological traits on four groundcover plants (*Lolium perenne, Potentilla* spp., *Trifolium repens* and *Frankinia* spp.) with the potential use in landscape. Results showed that *Frankinia* spp. and 75% lawn irrigation requirement bears the highest proline content with same amount (0.84 mg/g fresh weight) and on the other hand highest (32.61 mg/g fresh weight) and lowest (15.95 mg/g fresh weight) chlorophyll content was shown in control (irrigation treatments) and *Frankinia* spp., respectively. For total soluble carbohydrates content, highest amount (1.54 mg/g fresh weight) belongs to *Lolium perenne* whereas the lowest (0.79 mg/g fresh weight) belongs to *Trifolium repens*. *Trifolium repens* also had the most DPPH free radical-scavenging assay (75.05%) among species. In general, regulated deficit irrigation affects different biochemical characteristics of examined groundcover plants significantly, but as a managing method applying it to below the damaging point for landscape plants can be a good method for water consumption management in this manner.

Keywords: Antioxidant activity, Chlorophyll, Proline content, Total soluble carbohydrate content.

INTRODUCTION

For maintenance of urban landscaping, frequent watering increases the costs and does not collaborate with sustainable use of water resources. Low water-requiring or ‘‘water-wise’’urban landscaping is important policy tool in arid/semiarid areas in Iran. Water-wise landscaping can maintain acceptable appearance during drought when designed with more drought-tolerant plants than turf grasses and when irrigation is determined by plant water needs (Mee *et al.,* 2003). Appropriate species selection for water-wise landscaping can be improved with knowledge of drought tolerance and water stress response mechanisms among candidate species (Kjelgren et al., 2000).

Plants in nature are continuously exposed to several biotic and abiotic stresses. Among the environmental stresses, drought is one of the most severe stresses for plant growth and productivity. Water stress affects virtually every aspect of plant physiology and metabolism. Drought stress reduces both nutrient uptake by the roots and transport from roots to the shoots, due to restricted transpiration rates and impaired active transport and membrane permeability (Yuncai and Schmidhalter, 2005). Drought stress reduced dry matters of plants by reduction in the area of the leaf, height of plant and lateral stem number (Aliabadi Farahani *et al*., 2009).

Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, organic acids, calcium, potassium, etc. (Farooq *et al*., 2009). The production of reactive oxygen species (ROS) is linear with the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. The severity of this damage largely depends on the status of antioxidant systems, since plants develop antioxidants to remove toxic reactive oxygen species and protect the plant cells from lipid peroxidation and inactivation of enzymes that occur under stress (Smirnoff, 1993).

In recent years turf grass has been extensively used in urban greenspaces. Turf grass is a high water demanding plant and needs many operations for maintaining, it is essential to optimize its surface in dry regions. For partial replacement of lawns, the suitable option is ground cover plants that along with their easiness in maintaining conditions require less water.

In a comparative study between sport turfgarass and *Frankenia 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 lawns (Shooshtarian and Tehranifar, 2010). Acar and Var (2001) studied compatibility and ornamental potential of 19 ground cover plant species, endemic in Trabazon Province (Turkey). They recommended two species of *Sedum spurium* and *Thymus praecox* for application in urban green space because of their acclimation and high levels of coverage. Dou *et al.* (2004) recommended three species of ground cover plants native to Yunnan Province (China) among 205 species. Those three species were recommended regarding with growth habit and ornamental features to be used in urban landscape of tropical regions.

Water is becoming more and more rare resource in arid and semi arid regions of Iran that characterized by little rainfall, high solar radiation and high temperatures in the summer. In recent years, the normal seasonal droughts that have occurred in Iran have caused local and state government to enact water conservation ordinances. Urbanization and increases in population, however, are seriously threatening sustainable natural resources. At present, non-renewable groundwater resources are being depleted to an alarming extent. As high-quality water supply becomes limited, the use of saline water with high salt levels for landscape irrigation is being encouraged. Despite the voluminous research on the sensitivity of plants to water stress, relatively little attention has been paid to landscape plants. Several ornamental plants have long been considered to be very drought resistant but their actual water needs and the reasons behind their adaptations are not well known. Understanding the responses of plants to water deficits is led to establish methods for improving water use efficiencies, and to select appropriate plants and sites in planning landscape (Mohammadian *et al*., 2005). The aim of this study was to evaluate the physiological and biochemical responses of 4 ornamental ground cover species under drought stress.

MATERIAL AND METHOD

Plant material and experimental condition

This research was conducted using a factorial experiment based on a completely randomized design with regulated deficit irrigation at four levels (100, 75, 50, and 25% of lawn irrigation requirement) and three replicates to evaluate some biochemical and physiological indexes on four groundcover plants namely *Lolium perenne, Potentilla* spp., *Trifolium repens* and *Frankinia* spp. with the potential use in landscape. For this purposes transplants of three species were planted and *Lolium perenne* seeds were sowed in 48 plastic pots filled with simple horticultural soil and marketed peat. Water stress treatments started after two months of well water irrigation at the stage of establishment.

Leaf relative water content

Leaf Relative Water Content (LRWC) was calculated based on the method of Schonfeld *et al.*, (1988). Leaves were first removed from the stem and then weighed to obtain fresh mass (FM) at the harvest stage. In order to determine the turgid mass (TM), leaves were floated in distilled water inside a closed Petri dish for 6 hours. The leaf samples were weighed after gently wiping the water from the leaf surface with tissue paper, then the leaf samples were placed in an oven at 80℃ for 48 h, in order to obtain dry mass (DM). All mass measurements were made using an analytical scale, with a precision of 0.0001 g. Values of FM, TM and DM were used to calculate LRWC using the following equation:

$$LRWC (\%) = [(FM–DM)/(TM–DM)] \times 100.$$

Proline content

The proline content was estimated by the method of Bates *et al.* (1973).The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 1000 rpm. Supernatant was used for the estimation of proline content. The reaction mixture consisted of 2 ml supernatant, 2 ml ninhydrin acid and 2 ml of glacial acetic acid, which was boiled at 100º C for 1 h. After termination of the reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm. The proline concentration was determined using calibration curve. The results were calculated based on DW.

Chlorophyll content

Leaf samples were selected randomly from the plants and homogenized in a mortar in acetone. The extract was centrifuged at 5000 rpm for 5 min. Absorbance of the supernatant was recorded at 663, 645 and 450 nm spectrophotometrically (Techcomp 8500 II, South Korea). Chlorophyll (Chl) content was determined following the method of Arnon (1949).

Electrolyte leakage

Electrolyte leakage which is used to assess membrane permeability was determined according to Lutts *et al*. (1996). Leaf discs (1 cm in diameter) from two randomly chosen plants per plot were taken from the middle portion of youngest fully developed leaf and then were placed in individual stoppered vials containing 20 ml of distilled water after three washes with distilled water to remove surface contamination. After incubating the samples at room temperature on a shaker (150 rpm) for 24 h, the electrical conductivity (EC) of the bathing solution (EC1) was determined. The same samples were then placed in an autoclave at 121℃ for 20 min. and a second reading (EC2) was determined after cooling solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

Total soluble carbohydrate content

Total soluble carbohydrates were extracted according to a modified procedure described by Wardlaw and Willenbrink (1994). 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. Onetenth ml of alcoholic extract preserved in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid,W/W). The samples placed in boiling water bath for 10 minutes. The light absorption of the samples was estimated at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard and expressed as mg g^{-1}DW of leaves.

DPPH free radical-scavenging activies

The free radical scavenging ability was measured using the protocols described by Shimada *et al.* (1992) and Yoshiki *et al*. (2001). Briefly, an aliquot of 4 ml of the methanolic extract (4, 6 and 8 mg/ml) was added to 1 ml of 10 mM DPPH (2, 2-diphenyl^{-1}- picrylhydrazyl) solution freshly prepared in methanol. The mixture was left in the dark for 30 min, and decolorization of DPPH donated H+ was followed by measuring the absorbance at 517 nm. DPPH radical-scavenging activity was calculated from the absorption according to the following equation:

DPPH radical-scavenging activity % = [($A_{control}$ - A_{sample}) / Acontrol)] × 100.

Statistical Analysis

Analysis of variance (ANOVA) for all the variables was carried out using the JMP8 software. Treatment means were compared using the protected least significant difference (LSD) test at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Results of this experiment showed significant effects of water stress on different studied traits and also among different species. Mean comparison are shown in table 1.

Leaf relative water content

The results showed that water stress effect was significant ($p \leq 0.01$), but interaction effects were not significant. *Trifolium repens* (41.6%) had higher relative water content as compared to other species (Table 1). On the other hand, no signifiacant difference is observed in water stress treatments up to 50% of lawn requirement, and a sharp decline occurs from then. The rate of RWC in plant with high resistance against drought is higher than others. In other words, plant having higher yields under drought stress should have higher RWC. Decrease in RWC in plants under drought stress may depend on plant vigor reduction and have been observed in many plants (Liu *et al.,* 2002). Under water deficit, cell membrane subjects to changes such as penetrability and decrease in sustainability (Blokina *et al*., 2003).

Proline content

Water stress, species and their interaction effects were highly significant in proline content

Table 1. Mean comparison of studied traits in species and water stress levels.

		Proline (mg/g DW)	Carbohydrate (mg/g FW)	Chlorophyll content (mg/g FW)	DPPH scavenging (%)	RWC	EL
Plant species	*Lolium perenne*	0.54b	1.54a	31.3a	38.9c	32.7bc	29.5b
	Potentilla spp.	0.51b	1.38a	31.2a	57a	29c	12.4d
	Trifolium repens	0.54b	0.79b	27.99a	46.8b	41.6a	17.9c
	Frankinia spp.	0.89a	0.99b	15.9b	51.1ab	36.2ab	49.69a
Water stress	0	0.41d	1.2a	32.6a	7.8c	35.7a	20.2c
	25%	0.54c	1.4a	30.9a	53.8b	38.5a	22.7bc
	50%	0.65b	1.2a	21.4b	63.89a	39.3a	26.6b
	75%	0.84a	0.89b	21.5b	68.5a	26b	39.9a

Digits with different letters in each column are significantly different at P≤5%.

(p≤0.01). *Frankinia* spp showed the highest proline content (with 0.84 mg/g FW) among species. Under water stress, the highest amount of proline content were observed in 25% lawn requirement treatment and *potentila* spp. species (Fig. 1.).

Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses (Beltrano *et al*., 1997). The higher proline content could be due to enhance activity of ornithine aminotransferase (OAT) and pyrroline 5-carboxylate reductase (P5CR), the enzyme involved in proline biosynthesis as well as the inhibition of proline oxidase, proline catabolising enzymes (Debnath, 2008).

Chlorophyll content

Results from leaf chlorophyll content measurements showed a significant difference between water stress treatments and species (p≤0.01) and interaction effects was significant at 5% level. *Frankinia* spp showed the lowest chlorophyll content (15.9 mg/g FW) and 50% and 75% lawn requirement also showed the lowest chlorophyll amounts (Fig. 2). Our results are in agreement with Nyachiro *et al*. (2001), who described a significant decrease of chlorophyll a and b caused by water deficit in six *Triticum aestivum* cultivars. Under water deficit conditions, photosynthetic rate of all wheat cultivars was significantly reduced. It is well evident that reduction in photosynthetic rate occurs due to stomatal closure under water deficit conditions which may limit CO_2 diffusion into the leaves (Nyachiro *et al*., 2001).

Electrolyte leakage

Effects of drought stress, species and their interaction effects were significant on electrolyte leakage (p≤0.01). *Frankinia* spp. and *Potentila* sp scored the highest (49.6 %) and lowest (12.4%) respectively. 75% lawn requirement (39.9 %) also bears the highest electrolyte leakage water stress treatments. Electrolyte leakage increases linearly with the severity of drought stress in all 4 species.

Water stress can increase reactive oxygen species synthesis (ROS) that produce proteins, membrane lipids and photosynthetic pigments degradation with a loss of cell membrane stability

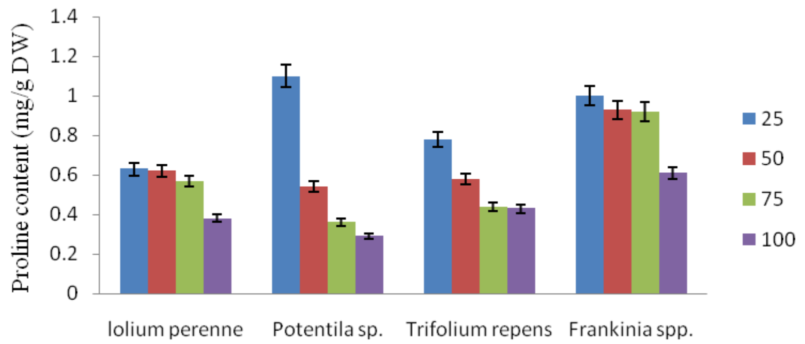

Fig. 1. Interaction effects of water stress and species on proline content.

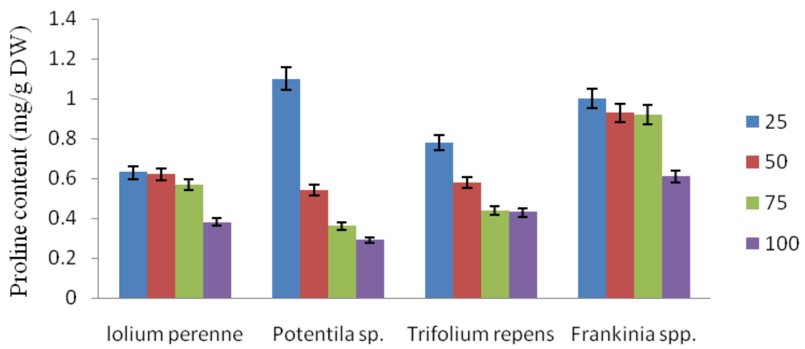

Fig. 2. Interaction effects of water stress and species on chlorophyll content.

(Navari-Izzo *et al.*, 1997; Beltrano *et al.*, 1997). Tolerance drought strategy could be associated to integrity cell membrane preservation and its rapid reparation (Oliver, 1991).

Total soluble carbohydrate content

According to the results of this study, applying water stress significantly affected total soluble carbohydrate content of plants significantly ($p \leq 0.01$). Interactions were not significant. Plant species are significantly different for this trait.The highest and lowest total soluble carbohydrate content (1.54 and 0.79 mg/g FW) was observed in *Lolium perenne* and *Trifolium repens*, respectively. The most water stress showed the lowest amount of this trait (0.89 mg/g FW). The tolerance mechanism in water-deficit may be associated with accumulation of osmoprotectants such as proline and soluble sugars. Accumulations of total soluble carbohydrates increase the resistance to drought stress in plants (Keyvan, 2010). Earlier reports mentioned that sugars protect the cells during drought by the following mechanism; the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes through hydrogen-bonding, thereby preventing protein denaturation (Al- Rumaih and Al- Rumaih, 2007).

DPPH free radical-scavenging activities

Water stress, species and their interaction effects were highly significant in DPPH free radical-scavenging activities ($p \leq 0.01$). The highest (68.5 %) and lowest (7.8 %) amounts of free radical scavenging activities were obtained from sever water stress and control treatments, respectively (Table 1). Fig 4. Interaction effects of water stress and species on DPPH free radical-scavenging assay.

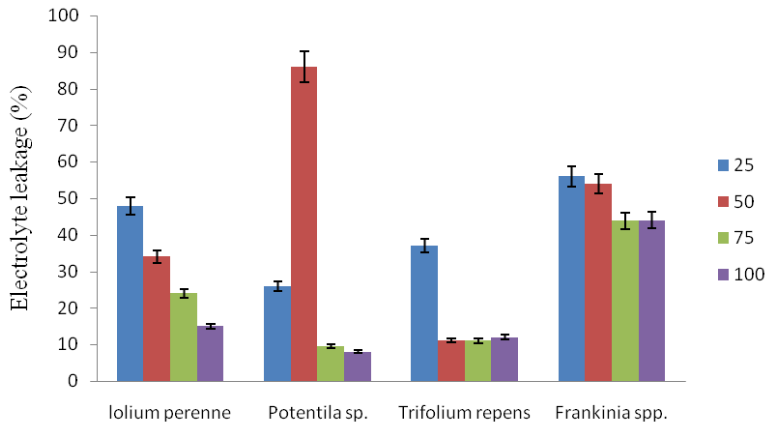

Fig. 3. Interaction effects of water stress and species on electrolyte leakage.

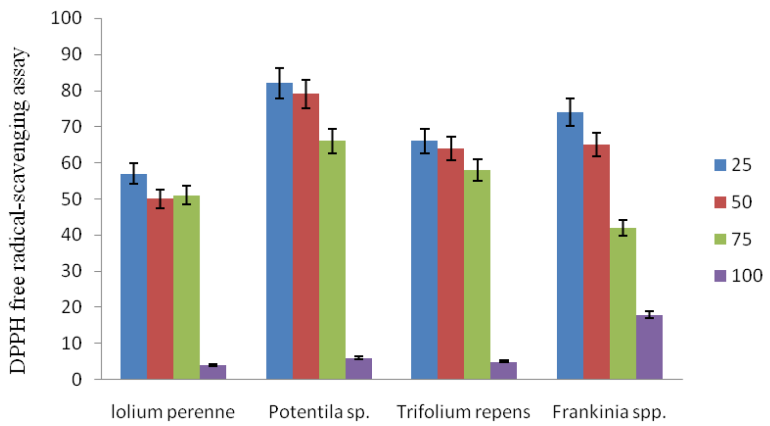

Fig. 4. Interaction effects of water stress and species on DPPH free radical-scavenging assay.

Water deficit stress induced the generation of reactive oxygen species (ROS) (Shao, 2008). It is now widely accepted that these cytotoxic ROS are responsible for various stress-induced damages to macromolecules mainly lipid membrane peroxidation which could be determined by measuring malon-dialdehyde (MDA). Enhancement in the production, ability and capacity of antioxidants may play an important role in metabolic stress tolerance and appeared to play a role in the protection of cellular machinery against damage by reactive oxygen species (Sairam and Srivastava, 2001; Guo *et al.*, 2006).

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