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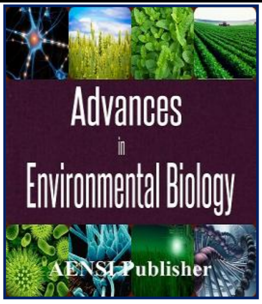

Differential Physiological and Morphological Responsesof Persian and Hybrid Petunia (*Petunia hybrida cv.* Sonja Pink) in Vegetative and Reproductive Growth Stagesunder Drought Stress

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ABSTRACT

Although low water use landscaping is becoming common in arid regions, little is known about drought tolerance and drought responses ofmany ornamental plants, especially herbaceous perennials. Petunia is a common flowering ornamental plant, largely produced for home-consumption and gardening.A factorial experiment based on a completely randomized design with irrigation at 3 levels (100, 70 and 40% FC) and Petunia hybrid (Persian and *Petunia hybrida cv.* Sonja Pink**)**in vegetative and reproductive growth stageswas conducted with 4 replicates. Results showed significant differences among measured traits. Obviously a great difference is demonstrated between Persian petunia and *Petunia hybrida cv.* Sonja Pink in almost all characteristics. Nearly all measured traits are affected by watering regimes. Interactions effects were also outstanding especially in shoot and root dry weights and flower characteristics. Drought stress decreased leaf area, plant height, and roots and shoots fresh and dry weight and root length significantly in both petunias.Highest and lowest values are mainly observed in well water (100% FC) and stress (40% FC) treatments, respectively. Flower number and lateral branches were reduced from 71.87 and 8.5 to 13 and 1, respectively.But main branches and flower diameter were less affected. It generally seems according to the results of this experiment, that petunia is asemi tolerant plant with moderatedrought resistance.

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INTRODUCTION

Landscape irrigation represents a major portion of the potable water used in arid and semi-arid regions. Throughout the aridIntermountain West, for example, 30–70% of all potable water isused to irrigate urban landscapes [6]. Water stress may arise as a result of two conditions,either due to excess of water or water deficit.Physiological effects of drought on plants are the reduction in vegetative growth; in particular shoot growth. While drought-tolerance mechanisms may allow plants tosurvive during drought, they do not insure the plants will have high visual quality.Growth reductions, for example, mayreduce the visual appeal of plants in the landscape. Even plantsthat have some degree of drought tolerance may still experience reductions in flower number, size and/or quality [8,10], wilting, and/or leaf burnwhen exposed to drought.Drought-adapted plants either totally avoid (i.e. completing their life cycle when water is not limiting) or tolerate drought.Drought avoiding species such as annuals may be minimallyuseful in low water use landscapes because they lose theirvisual appeal because of above-ground die back. In contrast,drought tolerant plants by maintaining normal above-groundappearance, despite being water stressed, are more likely to bevaluable in low-water landscapes where irrigation is infrequent.

While crop tolerance to salinity and drought has been givenconsiderable attention, fewer studies have dealt specificallywith ornamental plants. A number of growth controlling strategies using different approaches have been studied in recent years [4,7], especially involving the application of plant growth regulators [2]. One of the

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consequences of exposing plant to drier regimesin terms of plant growth is the production of smaller leaves andshorter internode sections and reductions in flower number, sizeand/or quality [12,8]. Differences in sensitivity todrought between different species and/or cultivars [16,9,14] and even between growthstages have been demonstrated for many plants [15,13].

Petunia is a common flowering ornamental plant, largely produced for home-consumption and gardening. In Iran it is also used as a main landscape flower for mild to warm seasons.

In conclusion to this point of view, the main aim of this study was to identify and compare different physiological and morphological responses of Persian and hybrid Petunia (*Petunia hybrida cv.* Sonja Pink) to drought stress invegetative and reproductive growth stages.

MATERIAL AND METHODS

This study was conducted in two individual experiments in vegetative and reproductive growth stages.2 petunia cultivars namely Persian and Sonja Pinkwere selected for a factorial experiment based on a completely randomized design with irrigation at 3 levels (100, 70 and 40% FC) with 4 replicates in vegetative and reproductive growth stages. Drought stress was imposed using %FC treatments.

Experiment 1: Vegetative Growth Stage:

Leaf surface was determined using a Li-3100 area meter (LI, Lincoln, Nebraska, USA). To measure RWC, fresh weight (FW) of two excised leaves per plant were weighed and placed in plastic bags in the dark with their petioles plunged in distilled water overnight to allow them to reach full turgor and, hence, to determine their turgid weight (TW). These leaves were then dried at 70 $^{\circ}$C for 24 h and their dry weight (DW) was recorded. Then RWC was calculated using the following equation:

% LRWC= (FW-DW)/ (TW-DW) × 100

Electrolyte leakage was calculated by following the standard method of Pinhero and Fletcher [5].

Leaf chlorophyll related SPAD units were determined using a SPAD-502 Chlorophyll Meter (Konica, Minolta, Tokyo) on 2 different leaves of each plant, and the mean value was calculated.

Root and shoot length was measured after picking up plants at the end of experiment period using a ruler. Leaf and branch numbers were also counted and recorded.

Roots, leaves, and stems of examined plants were weighted after picking up from experimental plots. They were then dried (70 $^{\circ}$C for 48 h), and dry weights were recorded.

Chlorophyll content was determined using Dere et al. [1]. 200 mg of fresh leaves was homogenized and extracted with 10 ml methanol 99% (v/v %). After this process absorbance was read at 666 and 653 nm chlorophyll a and b wavelength. Calculation equations used are as below:

CHL a= 15.65 A666 – 7.340 A653

CHL b= 27.05 A 653- 11.21 A666

CHL t= CHL a + CHL b

Experiment 2: Reproductive growth stage:

All the measured traits for vegetative growth stage are repeated in this experiment and some other traits such as main branch, lateral branch, flower number, flower diameter and flower tube length are also measured and recorded.

Results:

Results showed significant differences among measured traits. Obviously a great difference is demonstrated between Persian petunia and *Petunia hybrid cv.* Sonja Pink in almost all characteristics. Nearly all measured traits are affected by watering regimes.

Experiment 1: Vegetative growth stage:

Physiological and biochemical traits of petunia cultivars and drought stress levels in vegetative growth stage are shown in Table 1. According to the results, Persian petunia showed higher values in relative water content (67.46%), electrolyte leakage (30.73%) and carotenoid content (4.72 mg/g FW) compared to *Petunia hybrid cv.* Sonja Pink. Whereas it showed lower values in membrane stability index (0.7 %), chlorophyll a, b and total (20.51, 8.84 and 29.36 mg/g FW respectively) content.

Drought stress also imposed significant reduction in some measured traits. Relative water content and membrane stability index reduced by 20%. Electrolyte leakage, chlorophyll and carotenoid content increased along with stress severity (Table 1).

Table 1: Physiological and biochemical traits of petunia cultivars and drought stress levels in vegetative growth stage

	RWC (%)	MSI	EL (%)	CHLa (mg/gFW)	CHLb (mg/gFW)	CHLt (mg/gFW)	Carotenoid (mg/gFW)
Cultivar							
Persian	67.46a	0.69a	30.73a	14.87b	6.72b	21.6b	4.72a
Sonja Pink	62.53b	0.7a	29.13a	20.51a	8.84a	29.36a	1.12b
Drought							
100%FC	66.98b	0.76a	23.39c	15.77b	7.33b	23.11b	2.66a
70% FC	73.62a	0.71b	28.49b	16.03b	6.7b	22.73b	2.8a
40% FC	54.37c	0.62c	37.38a	21.28a	9.31a	30.6a	3.3a

Figures with different letters in each column are significantlydifferent at P≤5%.

Morphological indexes were shown to be noticeably different between two studied cultivars. Persian petunia was observed higher in leaf area (133.57 cm^2), root fresh (2.95 grams) and dry weight (0.14 grams) and shoot fresh (10.62 grams) and dry (0.64 grams) weight, leaf number (24.55), total root length (101859 cm) and branch number (4.44) in comparison to *Petunia hybrida cv.* Sonja Pink (Table 2).Drought stress caused a reduction on allmorphological indexes measured. Plant had no branches in 40 % FC.

Table 2: Morphological traits of petunia cultivars and drought stress levels in vegetative growth stage

	LA (cm^2)	RFW (g)	SFW (g)	RDW (g)	SDW (g)	L. No	PL (cm)	RL (cm)	RS	RD (mm)	B. No.
Cultivar											
Persian	133.57a	2.95a	10.62a	0.14a	0.65a	24.55a	2.81a	101859a	5076.36a	0.49b	4.44a
Sonja Pink	45.22b	1.71b	4.16b	0.07b	0.25b	15.77b	2.64a	10463a	6230a	0.53a	3.33b
Drought											
100%FC	142.09a	3.13a	11.06a	0.13a	0.64a	28.67a	3.33a	132275a	6691.67a	0.518a	6.50a
70% FC	105.81b	2.89a	9.18b	0.13a	0.55a	21.83b	2.86b	12302.70a	6932.93a	0.513a	5.17b
40% FC	20.28c	0.97b	1.93c	0.06b	0.16b	10.00c	1.98c	6498.50b	33350b	0.511a	0.00c

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

Among interaction effects, shoot fresh weight, leaf area and branch number are the traits most affected. According to these highest values of shoot fresh weight and leaf area is observed in Petunia hybrid cv. Sonja Pink and well water while the lowest are shown in Persian petunia and severe drought stress. Highest branch number is observed in Persian petunia (Fig. 1). From the results of this investigation, it can be concluded that the Persian petunia is moderately suited for commercial cultivation in water-deficit areas.

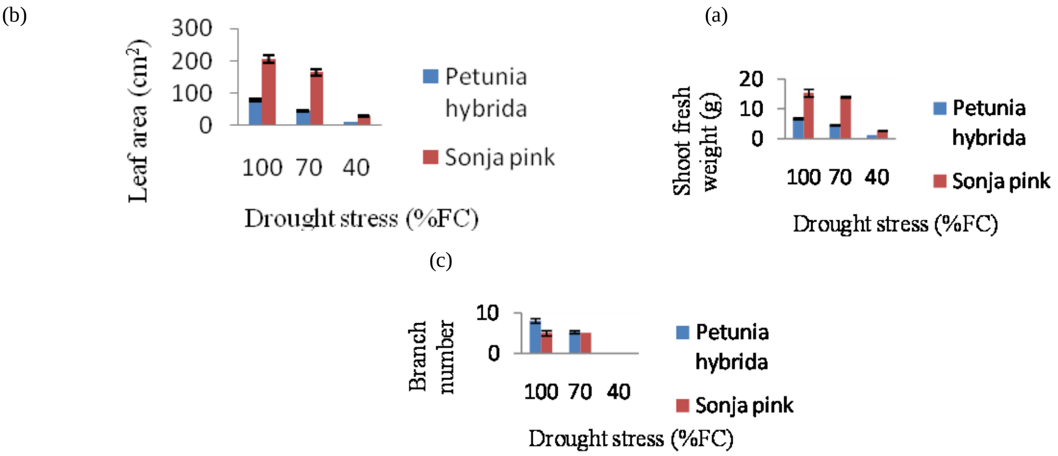

Fig. 1: Interaction effects on measured traits, shoot fresh weight (a), leaf area (b) and branch number (c)

Experiment 2: Reproductive growth stage:

Results show similar properties to vegetative growth stage as for two cultivars in the reproductive stage of growth (Table 3). Persian petunia shows a better look in relative water content (67.16%), electrolyte leakage (30.77%) and chlorophyll b content (18.35 mg/g FW) which is not significant, whereas *Petunia hybrida cv.* Sonja Pink is greater in membrane stability index (0.76%), chlorophyll a and total (28.46 and 46.6 mg/g FW) and carotenoid content (6.11 mg/g FW) (Table 3).Drought stress decreased relative water content and membrane stability index by 30 and 20 % respectively. Chlorophyll content (a, b and total) were observed greater in severe stress (70 and 40 % FC) and chlorophyll a/b ratio is not significantly different at any level.

Morphological indexes were shown to be noticeably different between two studied cultivars except for root diameter (Table 4). Persian petunia was observed higher all traits but only root surface. Drought stress caused a reduction on the majority of the measurements. Highest root diameter (0.493 mm) and root to shoot dry weight ratio was observed in 70 % FC and 40% FC treatments, respectively (Table 4). Main and lateral branch (5.91 and 5.5) and flower number (48.91) was observed higher in Persian petunia and flower diameter (5.63 cm) and flower tube length (2.82 cm) was higher in *Petunia hybrida cv.* Sonja Pink (Table 5).Drought stress decreased all of the measured traits significantly (Table 5). Lateral branch is the most decreased by 90 % (8.5 to 1) and flower diameter is the least reduction by 25 % (5.76 to 4.34 cm).

Table 3: Physiological and biochemical traits of petunia cultivars and drought stress levels in vegetative growth stage

	RWC (%)	MSI	EL (%)	CHLa (mg/gFW)	CHLb (mg/gFW)	CHLt (mg/gFW)	Carotenoid (mg/gFW)	CHLa/b
Cultivar								
Persian	67.16a	0.69b	30.77a	23.56b	18.35a	41.92b	3.85b	1.31b
Sonja Pink	65.41a	0.76a	23.4b	28.46a	18.12a	46.6a	6.11a	1.59a
Drought								
100%FC	73.62a	0.81a	19c	22.56b	16.36b	38.93b	4.18b	1.41a
70% FC	66.62b	0.71b	28.6b	28.42a	18.99a	47.41a	6.01a	1.49a
40% FC	58.62c	0.66c	33.66a	27.04a	19.39a	46.43a	4.74b	1.45a

Table 4: Morphological traits of petunia cultivars and drought stress levels in vegetative growth stage

	LA (cm^2)	RFW (g)	SFW (g)	RDW (g)	SDW (g)	SDW r/l	PL (cm)	RL (cm)	RS	RD (mm)
Cultivar										
Persian	716.95a	20.48a	103.64a	2.73a	10.4a	0.31a	34.92a	56309.4a	7334.6b	0.461a
Sonja Pink	177.66b	3.28b	32.33b	0.43b	3.52b	0.12b	21.33b	14801.1b	32916.3a	0.469a
Drought										
100%FC	682.12a	17.86a	112.64a	2.37a	12.22a	0.165c	38.25a	45546.9a	26706.6a	0.471b
70% FC	455.74b	13.65b	69.39b	1.91b	6.96b	0.221b	32.12b	44692a	25395a	0.493a
40% FC	204.06c	4.67c	21.92c	0.46c	1.7c	0.271a	14c	16426.8b	8274.8b	0.446b

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

Table 5: Flower characteristics measured in reproductive growth stage

	Main branch	Lateral branch	Flower No.	Flower diameter (cm)	Flower tube length (cm)
Cultivar					
Persian	5.91a	5.5a	48.91a	4.79b	2.35b
Sonja Pink	4.16b	3.67b	37b	5.63a	2.82a
Drought					
100%FC	6.75a	8.5a	71.87a	5.76a	2.96a
70% FC	4.37b	4.25b	44b	5.25a	2.67b
40% FC	4b	1c	13c	4.34b	2.13c

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

Among interaction effects, flower number, lateral branch and shoot dry weightare the traits most affected. According to the results recorded, highest values of shoot dry weightand flower number is observed in Petunia hybrid cv. Sonja Pink and well water while the lowest are shown in Persian petunia and severe drought stress (Fig. 2). Highest lateral branch number is observed in Persian petunia.

Discussion:

Water limitation has an impact on plant growth, although the exact effect may vary depending on theintensity of the water stress imposed [8]. Several authors suggested that, under stress, the osmotic effect is responsible for the aerial organ growth reduction. Root, shoot and leaf dry weight reduced with increased salinity water irrigation in *Asteriscus maritimus* plants. It seems that a reduction in whole plant fresh and dry weight in different organs is due to the limiting factor of photosynthesis.

A reduction in canopy area as an avoidance mechanism along with chlorophyll deterioration which usually occur in salinity stress, may be the main reason for this slight decrease. Visual symptoms of salt injury, such as burning leaf margin were observed in plants.

Enhanced electrolyte leakage was considered to be a symptom of stress-induced membrane damage and deterioration. There was significant difference in EL between treated plants and different cultivars.

Leaf relative water content (RWC) is an index representing theamount of water in the plant organs and shows the ability of a plant in maintaining water understress conditions [3]. So in a controlled environment for an experiment, themeasured RWC shows the response of a plant and the higher the measured amount, the greaterthe ability of a treatment for keeping water [3]. Results in this experimentshow a gradual linear decrease from 0 to 150 mM and a big collapse from then to 225 mM. Theseresults explain optimum resistance levels in *Trifolium* species to a mild salinity stress and sensitivityto sever stress. Our findings agree with Genhua and Denise which RWC of *Ceratostigma plumbagioides* decreased significantly from 78% to 64% as irrigation salinity increased and donot agree with Nader et al., on *Crithmum maritimum* which shows a very small decreasein root and shoot RWC with increasing salt concentration.

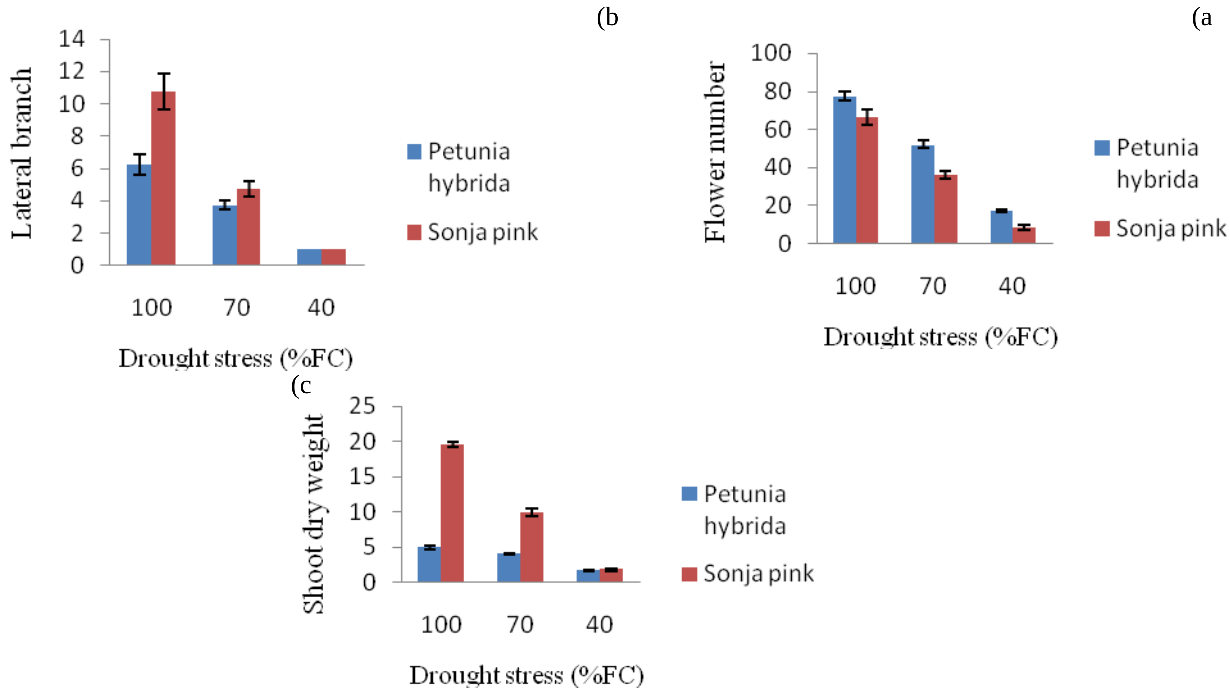

Fig. 2: Interaction effects on measured flowering stem traits, flower number (a), lateral branch (b) and Shoot dry weight (c)

Reduction in Water deficit may influenceflowering by inhibiting vegetative growth [8]. Hence, the reduction in flower characteristics under drought stress is probably due to a reduced vegetative growth of petunia. Water stress has decreased flower visual quality in both cultivars andgrowth stages.Leaf area under drought and saline stress can be considered as avoidance mechanisms, which minimize water losses when the stomata are closed, which happens to many species under osmotic stress.

This redistribution of dry matter in favor of the roots at the expense of shoots is probably due to the plantsneeding to maintain root surface area under drought conditions inorder to absorb water from the substrate. An advantage for the smaller surface area, as we can observein our experiment, is its contribution in reducing water consumption, since canopy transpiration is a function of the net sunshineenergy absorption and lower leaf area will reduce light interception [2].

Conclusion:

Drought stress was shown to be significant on different petunia cultivars and also growth stages. Results showed significant differences among measured traits. Obviously a great difference is demonstrated between Persian petunia and *Petunia hybrida cv.* Sonja Pink in almost all characteristics. But these are according to the properties of each cultivar. It generally seems according to the results that petunia is semi tolerant a plant with good drought resistance and SA could act as a useful and beneficial stress alleviator for reducing harmful stress effects.

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