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Research Article

EFFECT OF SALICYLIC ACID ON MORPHOLOGICAL AND ORNAMENTAL CHARACTERISTICS OF *PETUNIA HYBRIDA* AT DROUGHT STRESS

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ABSTRACT
A factorial experiment base on randomized complete block design with three replications carried out to investigate the effect of salicylic acid at enhancing stress tolerance in petunia plants during 1392. Factors were two petunia varieties (Sonja pink and an Iranian variety), two irrigation levels (70 and 40 percent of FC) and three salicylic acid doses (0, 1 and 2 mM). There were significant differences between varieties in response to different irrigation levels and salicylic acid doses. Flower number per plant, flower diameter, leaf area, shoot and root dry weight, biomass, chlorophyll content, stomatal conductance decreased by higher drought level. Electrolyte leakage enhanced by drought stress. Destructive effects of drought stress on petunia, reduced by applying salicylic acid reduced. Morphological and ornamental characteristics of flowers improved by higher doses of salicylic acid. Electrolyte leakage decreased using 2 mM of salicylic acid. Results showed that drought tolerance decreased by salicylic acid in both varieties. Best results gained by applying 2 mM of salicylic acid.

Keywords: *Drought Stress, Biomass, Salicylic Acid, Electrolyte Leakage, Stomatal Conductance*

INTRODUCTION
High quality water resources are limit in arid and semi-arid areas of the world. Mean precipitation of Iran is about 252 mm which is one third of global precipitation mean. Water deficit is one of the most important limiting factors in crop cultivation in Iran and drought stress is inevitable during plant growth season (Khodabande and Jalilian, 1997). Petunia is an annual ornamental plant belongs to Solanaceae family which originated in South America (Khalighi, 1997). Petunia is widely planted in urban landscape and it is necessary to control its water consumption by improving its drought resistance. Plants response to environmental stresses is different and depends on stress intensity and duration and time of stress occurrence (Bohnert and Jensen, 1996) and plant spices and growth stage (Mirfatah *et al.*, 2008; Sanchez *et al.*, 1998). Plants have different mechanisms to reduce stress effects. Water deficit results in lower plant growth and yield due to lower photosynthesis rate and leaf area (Miranzade and Emam, 2010). Cell membrane stability known as an indicator of drought tolerance in plants. Leaf relative water content is an appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit and electrolyte leakage (EL) is another indicator of cell injuries during drought stress (Foker *et al.*, 1998; Fu *et al.*, 2004). Osmoregulation is one of drought resistance mechanisms of plants (Izadi *et al.*, 2009). Applying exogenous compounds is one way to reduce destructive effects of abiotic stresses (Yuan *et al.*, 2008). Salicylic acid (SA) is a messenger molecule which plays a non-enzymatic anti-oxidant role in regulating plant physiological mechanisms during stress occurrence (Arfan *et al.*, 2007). Fresh and dry weight of root and shoot, stem diameter and leaves number of cucumber plant increased by spraying salicylic acid (Yildirim *et al.*, 2008). Samia *et al.*, (2009) reported that oxidative stress reduced by applying 0.15 mg of exogenous salicylic acid at saline condition. Soaking seeds and leaf spray of seedlings of melon by 0.1-1 mM acetyl salicylic acid reduced injury effects of drought stress in treated plants (Korkmaz *et al.*, 2007). Shoot growth of barley (Pancheva, 1996) and wheat (Shakirova, 2007), root length and lignin accumulation in cell walls of soya bean increased using salicylic acid (Al-Hakimi, 2008). Petunia is commonly panted in arid and semi-arid regions of Iran as a landscape plant,

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thus the experiment was conducted to investigate the effect of applying salicylic acid on petunia growth and ornamental characteristics in order to enhancing plant tolerance to drought condition.

MATERIALS AND METHODS

Seeds of two varieties of *Petunia hybrida* planted in trays which filled with coco peat at 2013 growing season. The experimental site located at 59°35´ E and 36°17´ N. Studied varieties were Sonja pink and Iranian variety of petunia. Coco peat had an acidity in the range of pH 5.8 to 6.5 and EC in range between 0.75 to 1.25 dS/m. Germination temperature of laboratory was between 24-29 °C (Vall, 2004). Seedlings moved to pots with 12 cm diameter, at 7-8 leaves stage. Pots filled with 1 loam: 1 sand: 1 leaf mold mixture. A factorial experiment base on randomized complete block design with three replication was carried out. Factors were two petunia varieties (Sonja pink and an Iranian variety), two irrigation levels (70 and 40 percent of FC) and three salicylic acid doses (2, 1 and 2 mM). The first salicylic acid (SA) treatment applied 45 days after transferring of seedlings to pots. This was the beginning of productive phase of plants. Leaves sprayed each 10 days, with salicylic acid until two sides of leaf completely wetted. Drought stress applied base on field capacity in each treatment.

Each pot weighted by a digital scale every day and water added up to determined level (base weight loss of each pot). Day and night temperature of greenhouse adjusted at 25 and 18°C with 65% relative humidity. Plants were treated for 30 days. At the end of 30 days ornamental characteristics of plants measured. Measured traits were: flower number in each plant, flower diameter, corolla tube, plant height, main and secondary shoots counted in 10 plants and average of the numbers subjected to analysis of variance. Root and shoot samples oven dried at 72°C for 48 hours for determining dry weight. Flower diameter and corolla tube measured using Callipers. Plant height measured applying ruler.

Leaf area measured using and leaf area meter (Li-Cor, Model Li-1300, USA). Chlorophyll content measured five time in each treatment by SPAD 502, Minolta, Japan. Stomatal conductivity determined with porometer device.

Electrolyte leakage estimated in five samples of each treatment using following equation (Lutts *et al.*, 1996):

$$EL = [(EL0/EL1) \times 100]\%$$

While EC0 and EC1 refers to primary and secondary electrical conductivity and EL refers to electrolyte leakage. At the end of growing time, pots emptied and fresh shoot and root weight measured by digital scale. Then samples oven dried at 72 °C for 48 hours and dry weight measured for each sample.

Data were analyzed applying JMP8 software. Comparison of means carried out using LSD test at 5% probability level.

RESULTS AND DISCUSSION

Results

Analysis of variance showed that all measured traits (except for electrolyte leakage) significantly affected by variety, irrigation level and salicylic acid doses ($p<0.01$) (table 1). All measured traits affected by interaction between variety and irrigation level. All measured traits except for chlorophyll content, EL and branch number significantly affected by interaction between variety and SA. All flower characteristics except for flower diameter, plant height and main and secondary shoots affected by interaction between irrigation level and salicylic acid dose. Interaction between variety, irrigation level and SA dose was significant in respect of flower number per plant, corolla tube, leaf area and root dry weight ($p<0.01$) (table 1).

Comparison between means showed that Sonja pink had higher amounts in all measured characteristics compare with Iranian variety (table 2). All plant characteristics decreased by 40% FC compare with 70% FC water level (table 2).

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Table 1: Analysis of variance of studied treatments on petunia hybrid

Source of variation	df	flower number per plant	Flower diameter	Corolla tube	Chlorophyll content	Plant height	Leaf area	shoot dry weight	root dry weight	Biomass	Electrolyte leakage	Sotmatal conductivity	Primary shoot number	Secondary root number
††C	1	**†1213.36	**5.36	**17.50	**1320.1	**1034.69	**1813690.7	**581.29	/13**25	**848.16	96/69**	0.03ns	**53.77	**18.77
I	1	0**4784.2	**5.84	**2.10	**1373.9	**1332.25	**1038225.01	**394.94	**15.21	**565.17	/25**1892	**803.73	**53.77	**18.77
SA	2	**2075.16	**4.95	**1.60	**859.1	**362.72	**262167.9	**69.62	**3.71	**105.41	709/72**	**317.67	**24.66	**26.00
C×I	1	42.25ns	*0.42	**.20	**164.6	**367.36	**484508.1	**229.42	**10.51	**338.19	*26.69	**157.70	**7.11	**4.00
C×SA	2	*76.05	**1.36	**.17	8.35Ns	**57.05	**91977.6	*15.15	**2.95	**30.62	10.72ns	**31.45	4.22ns	**8.22
I×SA	2	**247.05	ns 0.05	**.38	**84.31	8.16ns	**108656	*21.29	**1.24	**31.62	**69.50	*27.38	4.22ns	0.22ns
C×I×SA	2	*104.16	ns 0.03	**.17	ns31.45	12.5ns	**61601.7	7.43ns	**1.16	ns 13.98	1.38ns	4.07ns	1.55ns	0.66ns
error	24	258.66	0.80	.26	171.26	44.66	30698.5	51.04	1.49	59.03	84.00	66.86	16.66	11.33

*††C, I and SA abbreviations for variety, irrigation levels and salicylic acid doses. * and **significant at 5% and 1% probability level respectively, ns sot significant*

Table 2: Mean comparing for morphological and ornamental traits in *petunia hybrida*

Treatment	Flower number per plant	Flower diameter (cm)	Corolla tube (cm)	Chlorophyll content	Plant height (cm)	Leaf area (cm^2)	shoot dry weight (g/plant)	root dry weight (g/plant)	biomass (g/plant)	Electrolyte leakage	Stomatal conductivity	Primary shoot number	Secondary shoot number
Iranian variety	20.77b	4.71b	3.77a	50.51a	29.38a	588a	10.65a	1.99a	12.65a	32.11a	15.73a	5.22a	4.05a
Variety Sonja pink	32.38a†	5.48a	2.38b	38.40b	18.66b	139b	2.62b	0.33b	2.95b	28.83b	15.67a	2.77b	2.61b
irrigation													
70%FC	38.11a	5.5a	3.32a	50.63a	30.11a	533.42a	9.95a	1.81a	11.76a	23.22b	2043a	5.22a	4.05a
40%FC	15.05b	4.7b	2.83b	38.28b	17.94b	193.8b	3.33b	0.52b	3.84b	37.73a	10.98b	2.77b	2.61b
SA (mM)													
0	17.5c	4.66c	2.80c	38.92c	20.33c	260.16b	4.71b	0.72c	5.44b	36.16a	12.71c	2.83b	2.16b
1	26.16b	5.1b	3.13b	43.65b	23.66b	361.46b	7.24a	1.27b	8.51a	29.91b	14.65b	4.50a	3.66a
2	36.08a	5.54a	3.30a	50.80a	28.08a	496.16a	7.59a	1.49a	9.45a	25.33c	19.75a	4.666a	4.16a

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Table 3: Mean comparing for morphological and ornamental traits in *petunia hybrid* (two-fold interactions)

Treatment		Flower number per plant	Flower diameter (cm) r	Corolla tube (cm)	Chlorophyll content	Plant heigh (cm) t	Leaf area (cm^2)	shoot dry weight (g/plant)	root dry weight (g/plant)	biomass (g/plant)	Electrolyte leakage	Stomatal conductivity	Primary shoot number	Secondary shoot number
Irrigation*variety														
Sonja pink	70%FC	45a†	6a	2.7c	42.44b	21.55b	192.95c	3.41b	.43c	3.84c	22.44c	22.55a	3.35b	3b
	40%FC	19.77c	4.97b	2.06d	34.36c	15.77d	85.33c	1.83c	.22c	2.05d	35.22b	8.9b	2c	2.22c
Iranian variety	70%FC	31.22b	5.01b	3.94a	58.83a	38.66a	783.77a	16.49a	3.19a	19.68a	24c	18.31b	6.88a	5.11a
	40%FC	10.33d	4.42c	3.61b	42.2b	20.11c	302.22b	4.82b	.81b	5.63b	40.22a	13.04c	3.55b	3b
SA × variety (mM)														
Sonja pink	0	22.01c	5.3b	2.2e	33.38e	16.5f	100f	1.61d	.26d	1.88d	33.83b	13.73c	1.66d	2c
	1	34b	5.46b	2.38e	36.95d	18.33e	131.93e	2.78cd	.38d	3.16cd	28.33d	14.95c	2.83c	2.33c
	2	41.16a	5.7a	2.56d	44.88c	21.16d	185.5d	3.47c	.33d	3.8c	24.33e	18.53b	3.83b	3.5b
Iranian variety	0	16d	4.03d	3.4c	44.46c	24.15c	420.33c	7.81b	1.18c	9b	38.5a	11.7b	4b	2.33c
	1	18.33c	4.73c	2.88b	5035b	29b	591b	11.71a	2.16b	13.87a	31.5c	14.35c	6.16a	4.83a
	2	31b	5.38b	4.05a	56.73a	35a	752.82a	12.44a	2.64a	15.09a	26.33de	20.98a	5.5a	5a
SA (mM)*irrigation														
70%FC	0	25.83c	5.11c	3.18b	47.26b	26.66c	364c	6.97b	1.12c	8.09b	27d	16.83b	3.66b	3b
	1	37.66b	5.5b	3.33a	48.78b	30.16b	528.76b	11.3a	1.99b	13.29a	24e	18.75b	5.66a	4.33a
	2	50.83a	5.9a	3.45a	55.76a	33.5a	707.5a	11.58a	2.32a	13.9a	18.66f	24.71a	6.33a	4.83a
40%FC	0	9.16e	4.21e	3.41d	30.58d	14f	156.33e	2.46d	.33e	2.79d	45.33a	8.6d	2c	1.33c
	1	14.66f	4.7d	2.93c	38.51c	17.16e	194.16de	3.187cd	.55de	3.73cd	35.83b	10.55d	3.33b	3b
	2	21.33d	5.18c	3.16b	45.75b	22.66d	230.83d	4.33c	.65d	4.99c	32c	13.8c	3b	3.5b

Means with the same letters in each column are not significantly different

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Table 4: Mean comparing for morphological and ornamental traits in *petunia hybrid* (three-fold interactions)

Treatment			Corolla tube (cm)	Chlorophyll content	Plant Height (cm)	Leaf area (cm^2)	shoot dry weight (g/plant)	root dry weight (g/plant)	Biomass (g/plant)	Sotmatal conductivity	Secondary shoot number
SA(mM) *irrigation*variety											
Sonja pink		0	2.56f[†]	38.60e	20.33fg	140.66fg	1.98ef	.34f	2.33efg	20.03b	2.33de
	70%FC	1	2.73ef	41.20fe	21.66ef	178.53f	4.04cde	.55ef	4.59cde	21.50b	2.66cde
		2	2.80e	47.53cd	22.66e	259.66e	4.20cde	.41f	4.61cde	26.13a	4b
		0	1.83i	28.16h	12.66i	59.33h	1.24f	.19f	1.43g	7.43g	1.66ef
	40%FC	1	2.03h	32.70g	15.00h	85.33gh	1.51f	.20f	1.72fg	8.40fg	2def
		2	2.33g	42.23ef	19.66fg	111.33gh	2.74def	.26f	3.0efg	10.93ef	3bcd
Iranian variety		0	3.80c	55.93b	33.c	587.33c	11.95b	1.90c	13.85b	13.63de	3.66bc
	70%FC	1	3.93abc	56.36b	38.66b	879b	18.56a	3.43b	22a	16cd	6a
		2	4.10a	64.20a	44.33a	1155.33a	18.96a	4.23a	23.20a	25.30a	5.66a
		0	3.00d	33.g	15.33h	253.33e	3.68cdef	.47f	4.15def	9.76fg	1f
	40%FC	1	3.83bc	44.33de	19.33g	303de	4.85cd	.90de	5.75cd	12.70e	4b
		2	4.00ab	49.26c	25.66d	350.33d	5.93cc	1.05d	6.98c	16.66c	4b

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Flower diameter, flower diameter, corolla tube, chlorophyll content, plant height, leaf area, root and shoot dry weight, biomass, stomatal conductivity and main and secondary shoots increased by applying 2 mM salicylic acid compare with 0 and 1 mM doses (table 2). The lowest and highest electrolyte leakage observed by 2 and 0 mM salicylic acid respectively. There was no significant difference between applying 1 and 2 mM of salicylic acid in respect of root dry weight, biomass and primary and secondary shoot number (table 2).

The highest and lowest flower number and diameter belonged to Sonja pink at 70% Fc and Iranian variety at 40% FC correspondingly. The highest and lowest corolla tube, chlorophyll content, plant height, leaf area, root and shoot dry weight, biomass and primary and secondary shoots number belonged to Iranian variety at 70% FC and Sonja pink at 40% FC respectively. The highest and lowest electrolyte leakage observed in Iranian variety at 40%FC and Sonja pink at 70% FC respectively (table 3).

The highest and lowest flower number and diameter produced by Sonja pink which sprayed with 2mM salicylic acid and Iranian variety with zero mM salicylic acid (table 3). The highest corolla tube, chlorophyll content, plant height, leaf area, root and shoot dry weight and biomass, stomatal conductivity, primary and secondary shoots produced by Iranian variety and 2 mM salicylic acid while the lowest belonged to Sonja pink and zero level of salicylic acid. The highest and lowest electrolyte leakage observed for Sonja pink with zero level of salicylic acid and Iranian variety with 2mM salicylic acid (table 3).

The highest and lowest flower number and diameter, corolla tube and chlorophyll content, plant height, leaf area, root and shoot dry weight, biomass, stomatal conductivity and primary and secondary shoots belonged to 70%FC and 2 mM salicylic acid and40% FC and zero dose of salicylic acid respectively.

The highest and lowest EL observed for 40% FC with zero dose of SA and 70% FC and 2 mM SA correspondingly (table 3). The highest and lowest flower number and diameter produced by Sonja pink at 70% FC and 2 mM SA, and Iranian variety at 40% FC and 0 mM SA (figure 1 and 2).

The highest and lowest corolla tube, chlorophyll content, plant height, leaf area, root and shoot dry weight and biomass, primary and secondary shoot number belonged to Iranian variety at 70% FC and 2 mM SA and Sonja pink at 40%FC and 0 mM SA (table 4, figure 3). The highest and lowest EL observed in Iranian variety at 40% FC and 0 mM SA and Sonja pink at 70% FC and 2 mM SA correspondingly (figure 4).

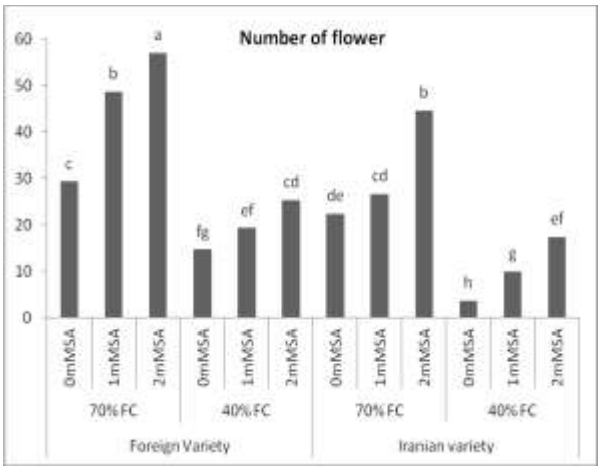

Figure 1: flower number as affected by interaction between variety, irrigation level and SA doses

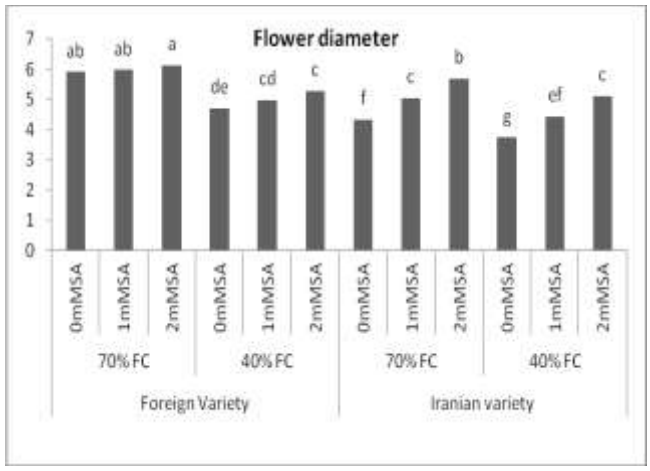

Figure 2: flower diameter as affected by interaction between variety, irrigation level and SA doses

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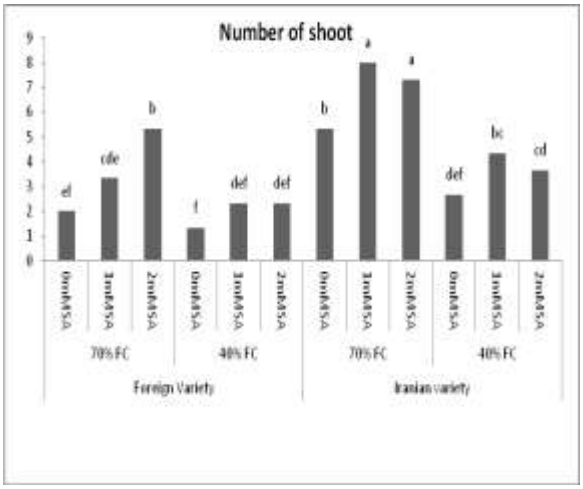

Figure 3: primary shoot number as affected by interaction between variety, irrigation level and SA doses

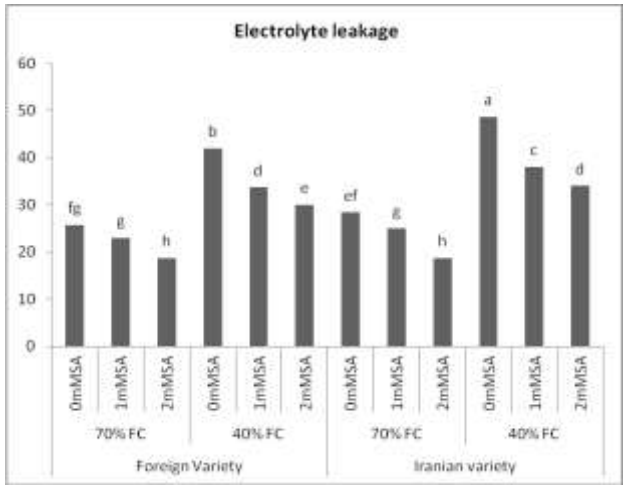

Figure 4: EL as affected by interaction between variety, irrigation level and SA doses

Discussions

Different varieties showed different responses to drought stress and applying of SA as expressed at results. Comparing these two varieties is hard due to their inherent differences in morphological and ornamental traits. But the lower EL of Iranian variety at drought condition showed its higher cell membrane stability compare with Sonja pink. In both varieties, drought results in lower amount of measured characteristics. EL of both varieties increased by 40% FC compare with 70% FC. Cell membrane is the first part of cells which injure by drought stress (Inze and Van Montague, 1995). Drought stress result in oxidative stress which enhances reactive oxygen species production and storage in plant cells. Thus cell walls fatty acids oxidation occurs and cell wall stability will loss ((Inze and Montague, 1995).

Leaf area and shoot dry weight decreased by drought stress. Drought results in lower cell growth and decreases cell production by meristems (Tradieu *et al.*, 2000). Low leaf area results in low photosynthesis rate. Dry material production is directly depending to photosynthesis rate. Thus lower amount of leaf area will result in lower dry material accumulation in plants (Porwanto, 2003). Stomatal conductivity reduced by drought in both varieties. Drought stress results in abscisic acid (ABA) increase in leaves. ABA stimulates the closure of stomata in order to reduce water loss (Abdul *et al.*, 2009). Flower number and diameter and corolla tube decreased by drought stress which were in agreement with Bayat *et al.*, (2010).

All petunia characteristics improved and EL decreased, using SA in both irrigation levels. Other researchers reported the same results on cucumber (Bayat *et al.*, 2012), melon (Korkmaz *et al.*, 2007), tomato and bean (Senaranta *et al.*, 1999). Applying SA enhances accumulation of polyamines like putrescine, spermine and spermidine in plants. Higher amount of polyamines results in higher cell membrane stability (Nemeth *et al.*, 2002). In this experiment, applying SA resulted in higher chlorophyll content at drought condition. The same results reported in cucumber (Bayat *et al.*, 2012). SA prohibits auxin and cytokinin loss in plants and thus enhances cell division and plant growth. SA keeps photosynthetic aspects like chlorophyll content, at proper level and thus help plants to well growth and develop (Hayat *et al.*, 2010).

Morphological characteristics like leaf area, plant height, root and shoot dry weight, biomass, flower number and diameter and primary and secondary shoot numbers enhanced by applying SA compare with 0 mM SA, at drought condition. At drought condition, leaf number of violet and gloxinia enhanced by 10% using SA (Martin-Mexand and Larqué-Saavedra, 2001). Applying foliar spray of SA resulted in higher root and shoot fresh weight, root and shoot dry weight, stem diameter and leaf number of cucumber (Yildirim *et al.*, 2008) and maize (Khodary *et al.*, 2004) at salinity. Stem diameter, biomass,

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plant height and leaves number of cucumber enhanced using SA at drought condition (Bayat *et al.,* 2012). SA regulate plant growth and cell division via other hormones like auxin, cytokinin, gibberellin and ABA. SA results in higher cell division in meristems and enhances root length (Shakirova *et al.,* 2003). Salicylic acid controls photosynthesis system, photosynthesis amount, pigment content and stomatal conductivity and regulates these procedures for appropriate growth and development (Popova *et al.,* 2009, Steven *et al.,* 2006, El-Tayeb, 2005, Kormkaz *et al.,* 2007).

Conclusion

Results showed that morphological and ornamental characteristics of both varieties affected by drought significantly. Applying salicylic acid improved measured traits at drought condition. Iranian variety showed higher resistance to drought compare with Sonja pink. In both varieties the highest drought resistance observed by applying 2 mM salicylic acid.

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