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Effect of foliar application with salicylic acid on two Iranian melons (*Cucumis melo* L.) under water deficit

Hossein Nastari Nasrabadi[1], Hossein Nemati[1]*, Mohammad Kafi[2] and Hossein Arouei[1]

[1]Department of Horticultural Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

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Scarcity of water is a severe environmental constraint to crop production in arid and semi arid region. Salicylic acid (SA) plays an important role in the regulation of plant growth in response to environmental stresses. In this study, the effect of SA (0, 70 and 100 ppm) was investigated on growth and yield of two Iranian melons (Khatooni and Ghasri cultivars) under drought stress (W100%, W80% and W60%). Experimental design was a split factorial based on complete block design with four replications. Results showed average fruit weight, yield and fruit ripening duration were decreased by increasing water restriction but total soluble solid (TSS) and proline content increased. Salicylic acid increased chlorophyll content (SPAD), fruit ripening duration and TSS than control. Different water treatments and salicylic acid levels had no significant effects on number of fruit per plant.

Key words: Drought stress, melon, salicylic acid, total soluble solid (TSS), yield.

INTRODUCTION

Drought stress cause adverse effects on plant growth and productivity of crops. Drought stress cause an increase of solute concentration in environment, leading to an osmotic flow of water out of plant cells (Taheri-Asghari et al., 2009). Plants respond to environmental stress by induction of various morphological, biochemical and physiological responses. Sayyari et al. (2013) reported that drought stress decreased fresh weight, dry weight, leaf area and relative water content (RWC) but increased proline content in lettuce. Salicylic acid, a ubiquitous plant phenolic compound, has been reported to regulate a number of processes in plants (Hayat et al., 2008). It can improve plant growth under drought conditions and other stresses (Senaratna et al., 2000). The salicylic acid (SA) increased the leaf area and dry matter production in corn and soybeen (Khan et al., 2003) and *Brassica junca* (Fariduddin et al., 2003). Exogenous application of SA improved the drought tolerance of wheat (Horvath et al., 2007). Jamali et al. (2011) reported that SA increased root and shoot fresh weight and yield of strawberry. Ghaderi et al. (2015) reported that SA increased leaf area, leaf number, proline content and yield in strawberry under drought stress. In another study on tomato under drought condition, SA increased proline content, RWC, SPAD and decreased electrolyte leakage (Hayat et al., 2008). This experiment was conducted to asses if SA could ameliorate the adverse effect of water deficit on melons.

*Corresponding author. E-mail: nematish@yahoo.com

Table 1. Mean squares from the analysis of traits.

S.V	DF	L.A	SPAD	P	NFPP	F.W	F.R	TSS	Y
R	3	0.02^{ns}	1.14^{ns}	0.0006^{ns}	0.20^{ns}	0.19^{ns}	2.78^{ns}	0.21^{ns}	20.22*
W	2	8.80**	76.98**	0.5857**	1.43^{ns}	11.14**	292.68**	4.88*	704.43**
E_w	6	0.02	1.39	0.0011	0.56	0.56	0.51	0.88	4.15
V	1	0.20^{ns}	15.13**	0.0328**	0.35^{ns}	0.03^{ns}	280.06**	17.50**	10.63^{ns}
SA	2	2.00**	46.93**	0.0321**	1.06^{ns}	0.55^{ns}	261.43**	5.72**	2.14^{ns}
W V	2	0.06^{ns}	0.55^{ns}	0.0075*	0.43^{ns}	0.01^{ns}	0.35^{ns}	0.95^{ns}	6.03^{ns}
W SA	4	0.10^{ns}	098^{ns}	0.0034^{ns}	0.24^{ns}	0.10^{ns}	6.78**	0.51^{ns}	2.76^{ns}
V SA	2	0.06^{ns}	0.96^{ns}	0.0011^{ns}	0.06^{ns}	0.10^{ns}	8.43**	0.21^{ns}	5.07^{ns}
W V SA	4	0.03^{ns}	0.49^{ns}	0.0002^{ns}	0.20^{ns}	0.08^{ns}	3.66*	0.15^{ns}	2.67^{ns}
E_{WvSA}	45	0.07	0.82	0.0018	0.50	0.42	1.25	0.36	3.06

** and * significant at 1% and 5% level respectively and ns no significant.

MATERIALS AND METHODS

In order to evaluate the effects of SA under drought stress on two Iranian melons, a split factorial experiment based on complete block design with four replications was conducted in Torbat-e-jam (Longitude: 60˚48´, latitude: 35˚ 31´, altitude: 928 meters, with semi-arid climate, hot summers and cold winters) on sandy-loam soil field. Water treatment (W) in 3 levels (100, 80 and 60% water requirement) was considered as a main plot. SA (0, 70 and 100 ppm) with Khatooni (V1) and Ghasri (V2) cultivars were considered as a sub plot in a factorial design. Seeds of melon were planted in 1 may 2014. Six meters distance existed between every main plot and 3.5 m was between sub plots. Plants irrigated every 7 days. The first SA spraying was carried out when fruits had 10 cm length and after 20 days the second spraying was done. After one week leaf samples were collected for measuring traits.

Water requirement calculated base on a thirty years period weather of Torabt-e-jam, soil and water analysis and date of planning by OPTIWAT software was planned by Alizadeh professor of irrigation (Alizadeh and Kamali, 2009). Water requirement was calculated 9640 m^3/ha. In addition daily water requirement was calculated and the amount required for 7 days was put together and the water needed was measured by a volume counter. In this experiment leaf area (L.A), SPAD, proline content (P), number of fruit per plant (NFPP), average fruit weight (F.W), fruit ripening (F.R), total soluble solid (TSS) and yield (Y) were measured. The chlorophyll in the fresh leaf samples was measured by using Minolta chlorophyll meter (SPAD-502-Minolta, Japan). 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 10,000 rpm. The supernatant was used for the estimation of the proline content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 6 ml of toluene, and absorbance was read at 520 nm. Fruits harvested after ripening and weighed for every plot. Analysis of variance was carried out using Minitab software and Duncan's multiple range test calculated at 5% level of probability.

RESULTS AND DISCUSSION

Analysis of variance showed (ANOVA) that water treatments had significant effects on all traits unless NFPP. SA and cultivar had significant effects on SPAD, F.R, TSS and proline content at 1% level probability (Table 1). Results showed leaf area was decreased by increasing water restriction. Maximum (4.58 m) and minimum (3.43 m) leaf area were recorded by $W_{100\%}$ and $W_{60\%}$ respectively (Table 2). SA increased leaf area than control and the maximum leaf area (4.18) was obtained by SA_{100} (Table 2). There was no significant difference between two cultivars but Ghasri cultivar had more leaf area than Khatooni cultivar (Table 2). Interaction effects between water treatments with cultivars and with SA were not significant (Table 1) anyway the maximum leaf area was recorded by W_{100} with V_2 combination (4.58 m) (Table 3) and W_{100} with SA_{100} combination (4.96 m) (Table 4). Leaf area reduced by increasing water restriction, our results as well as Ghaderi et al. (2015), Barzgar et al. (2011) and Hayat et al. (2008). Growth is one of the sensitive physiological processes to drought, because cell expansion only occurs when turgor pressure is greater than cell wall pressure (Shao et al., 2008). In drought conditions, plants close their stomata to prevent the transpiration water loss (Mansfield and Atkinson, 1990). They may result in response to decrease in leaf water potential (Ludlow and Muchow, 1990). Also it decreases the inflow of CO_2 into the leaves, so CO_2 assimilation decreased (Shao et al., 2008).

Mean square of simple effects (Table 2) showed that the highest chlorophyll content was seen in $W_{100\%}$ (47.21) and SA_{100} (46.73). There was significant difference between two cultivars, Ghasri cultivar had more SPAD (45.64) than Khatooni cultivar (44.73) (Table 2). SA increased SPAD in all treatments than controls (Table 4). Our results were in agreement with Singh and Usha (2003) and Elizabeth and Munne-Bosch (2008). SA probably prevents from action of chlorophyll oxidase enzymes therefore it will be impediment chlorophyll breakdown, for this respect increased photosynthesis.

Proline content increased by drought stress, the maximum proline content was obtained by $W_{60\%}$ (1.558 mgr/gr fw) also proline increased by increasing amount of SA (Table 2). There was significant difference between

Table 2. Means comparison of simple effects for traits.

Treatment	L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
$W_{100\%}$	4.58^a	47.21^a	1.249^c	4.00^a	4.09^a	93.54^a	11.57^b	29.533^a
$W_{80\%}$	3.67^b	44.55^b	1.365^b	4.08^a	3.52^b	89.54^b	12.25^a	25.347^b
$W_{60\%}$	3.43^c	43.80^b	1.558^a	3.63^a	2.73^c	86.58^c	12.43^a	18.785^c
V_1	3.80^a	44.73^b	1.369^b	3.97^a	3.47^a	87.92^b	11.59^b	24.939^a
V_2	3.96^a	45.64^a	1.412^a	3.83^a	3.43^a	91.86^a	12.58^a	24.171^a
Control	3.61^c	44.01^c	1.360^b	3.71^a	3.33^a	86.46^c	11.71^b	24.629^a
SA_{70}	3.89^b	44.81^b	1.380^b	3.88^a	3.39^a	90.17^b	11.91^b	24.226^a
SA_{100}	4.18^a	46.73^a	1.431^a	4.13^a	3.62^a	93.04^a	12.64^a	24.810^a

Means in the same column with different letters differ significantly at 0.05probablitiy level according to Duncan's multiple rang.

Table 3. Means comparison of interaction effects between water treatments and cultivars for traits.

Treatment		L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
W_{100}	V_1	4.58^a	46.65^b	1.231^d	3.92^{ab}	4.11^a	91.50^b	10.90^e	29.415^a
	V_2	4.64^a	47.77^a	1.267^d	4.08^{ab}	4.06^{ab}	95.58^a	12.25^{bc}	29.651^a
W_{80}	V_1	3.60^b	44.02^d	1.359^c	4.25^a	3.53^b	87.50^d	11.73^d	26.232^b
	V_2	3.74^b	45.08^c	1.371^c	3.92^{ab}	3.50^b	91.56^b	12.77^a	24.463^c
W_{60}	V_1	3.33^c	43.52^d	1.518^b	3.75^{ab}	2.76^c	84.75^e	12.15^{cd}	19.171^d
	V_2	3.53^{bc}	44.08^d	1.599^a	3.50^b	2.71^c	88.42^c	12.71^{ab}	18.399^d

Means in the same column with different letters differ significantly at 0.05 probablitiy level according to Duncan's multiple rang.

two cultivars, Khatooni cultivar had less (1.369 mgr/gr fw) proline than Ghasri cultivar (1.412 mgr/gr fw) (Table 2). Interaction effects between water treatments and cultivars were significant. The maximum proline content (1.599 mgr/gr fw) recorded by $W_{60\%}$ with V_2 combination (Table 3). Interaction effects between W and SA were not significant anyway in all treatments SA increased proline accumulation (Table 4). Because SA acts as a signalling molecules to active the signalling cascades by ABA, H_2O_2 and Ca^{2+}. Theses cascades then active the synthesis of specific protein kinases and active more responses such as changes in gene expression. Also changes in plant metabolism including synthesis and accumulation antioxidants and osmoprotectants such as proline (Farooq et al., 2009).

Analysis of variance (Table 1) showed all of the treatments were not significant on NFPP but only water treatments had significant effects on fruit weight. The maximum (4.09 Kg) and minimum (2.73 Kg) fruit weight were obtained by $W_{100\%}$ and $W_{60\%}$ respectively. In this study distinguished SA had no significant effect on fruit weight anyway SA increased fruit weight (Table 2). Ghaderi et al. (2015) reported that SA increased fruit weight in strawberry.

Fruit ripening was decreased by increasing water stress. The maximum (93.54 days) and minimum (86.58 days) fruit ripening period was recorded by $W_{100\%}$ and $W_{60\%}$ respectively (Table 2). Between cultivars was significant difference, Ghasri cultivar late ripening (91.86 days) than Khatooni Cultivar (87.92 days) (Table 2). Fruits were treatments with SA more lately ripening than control and there were significant difference between treatments (Table 2). Interaction effects between water treatments and SA were significant. The maximum period (95.88 days) of fruit ripening was recorded by $W_{100\%}$ with SA_{100} combination and in all combinations fruit ripening increased as the concentration of SA increased (Table 4). The same results were reported by Lolaei et al. (2012). Probably SA inhibited ethylene production in fruits. Many researchers proposed the role of SA as an antagonist to ethylene action (Marissen et al., 1986; Leslie and Romani, 1988; Shafiee et al., 2010). Analysis of variance (Table 1) showed that water treatments had significant effects on TSS ($P \leq 0.05$). $W_{60\%}$ had more TSS (12.43%)

Table 4. Means comparison of interaction effects between water treatments and SA for traits.

Treatment		L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
W_{100}	Control	4.15^c	45.70^c	1.222^e	3.88^{ab}	4.05^{ab}	91.13^c	11.03^e	30.31^a
	SA_{70}	4.62^b	46.79^b	1.256^e	4.00^{ab}	3.88^{ab}	93.63^b	11.38^{de}	28.92^a
	SA_{100}	4.92^a	49.14^a	1.268^e	4.13^a	4.33^a	95.88^a	12.31^{abc}	29.36^a
W_{80}	Control	3.48^d	43.73^d	1.330^d	4.00^{ab}	3.38^{bcd}	86.00^d	11.81^{cd}	24.79^b
	SA_{70}	3.60^d	44.11^d	1.359^d	3.88^{ab}	3.53^{bc}	90.00^c	12.00^{bcd}	25.29^b
	SA_{100}	3.93^c	45.81^c	1.406^c	4.38^a	3.66^{ab}	92.63^b	12.94^a	25.96^b
W_{60}	Control	3.19^e	42.61^e	1.529^b	3.25^b	2.56^e	82.25^e	12.28^{abc}	18.79^c
	SA_{70}	3.44^{de}	43.54^d	1.526^b	3.75^{ab}	2.76^{de}	86.88^d	12.34^{abc}	18.47^c
	SA_{100}	3.66^d	45.25^c	1.619^a	3.88^{ab}	2.87^{cde}	90.63^c	12.66^{ab}	19.10^c

Means in the same column with different letters differ significantly at 0.05 probablitiy level according to Duncan 's multiple rang.

and $W_{100\%}$ (Table 2). According to the results V_2 had more (12.58%) TSS than V_1 (11.59). The SA_{100} significantly increased (12.64%) TSS than SA_{70} (11.91%) and control (11.71%) and wasn't seen significant difference between SA_{70} and control (Table 2). Our results were in agreement with Karlidag et al. (2009) and Javaheri et al. (2012).

Between treatments only water treatment had significant effect on yield. The yield decreased by water restriction. The highest yield was obtained by $W_{100\%}$ (29.533 t/ha) (Table 2). Our results were in agreement with Barzgar et al. (2011); Ghaderi et al. (2015); Hayat et al. (2008); Mirabad et al. (2013). There was no significant difference between SA treatments anyway SA_{100} had more yield (24.810 t/ha) than SA_{70} and control (Table 2). According to the analysis of variance (Table 1), there was no significant difference between two cultivars. Interaction effects between water treatments and cultivar showed that in $W_{100\%}$ treatments V_2 had more yield than V_1 but by increasing water stress V_1 had more yield than V_2 (Table 3). Results showed by increasing drought stress proline content increased especially in V_2 (Table 3). It is concluded that the tolerance of V_1 is more than V_2 to drought stress genetically.

Conclusion

Iran has high genetic variation of melon. More study need to identify tolerance genotype to breeding programs. SA increased level of antioxidant system both under drought stress and without stress conditions. We are suggesting evaluating different levels of SA to increase yield and quality for melon.

Conflict of Interest

The authors have not declared any conflict of interest.

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