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Assessment of Salicylic Acid Impacts on Seedling Characteristic of Cucumber (*Cucumis sativus* L.) under Water Stress

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Abstract

Impacts of various concentrations of salicylic acid (SA) on cucumber (*Cucumis sativus* L.) seedling characteristic were evaluated under different water stress levels by using a factorial arrangement based on completely randomized design with three replications at experimental greenhouse of Ferdowsi University of Mashhad, Iran. The studied factors included three water deficit levels (100% FC, 80% FC, and 60% FC) considered as first factor and five levels of SA concentrations (0, 0.25, 0.5, 0.75, and 1 mM) as second factor. Results showed that foliar application of SA at the highest concentration enhanced leaf area, leaf and dry weight while decreased stomatal conductance under high level of water deficit stress. Though, severe water deficit stress sharply raised the SPAD reading values. In general, exogenous SA application could develop cucumber seedling characteristic and improve water stress tolerance.

Keywords: antioxidants, leaf area, salicylic acid, stomatal conductance, water stress

Introduction

Water is the major factor limiting crop yields in numerous regions of the world. Even where water for irrigation is currently abundant, there are increasing concerns about future accessibility (Gregory *et al*., 2000). Water deficit affect virtually every aspect of plant physiology and metabolism. In addition, water stress affect the water relations of a plant on the cellular as well as whole plant level causing specific as well as unspecific reactions, damages and adaptation reactions (Beck *et al*., 2007). Cucumber (*Cucumis sativus* L.) is a vegetable crop of the *Cucurbitaceae* family and greenhouse's production is popular in many areas of the world (Terashima *et al*., 1994). Cucumber is a primary source of vitamins and minerals for human body but its caloric and nutritional value is very low (Wang *et al*., 1997). Cucumber plant is extremely sensitive to water deficit stress (Liu *et al*., 2009). Leaf growth, photosynthetic rate of cucumber were sharply declined by a decline in IAA and cytokinin contents, rather than ABA accumulation under water deficit conditions (Pustovoitova *et al*., 2005).

The environmental stress stimulates the disproportionate production of reactive oxygen species (ROS) such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) in plants (Liu *et al*., 2009). Plants respond to stresses were performs by the synthesis of signaling molecules (Hayat *et al*., 2010). These signaling molecules activate a range of signal transduction pathways, some of which relieve the plant to overcome the stresses (Jing-Hua *et al*., 2008). Salicylic acid (SA) plays a fundamental role in water stress tolerance and considerable interests have been focused on SA due to its ability to induce a protective impact on plants under water stress (Azoon and Youssef, 2010).

The role of SA as a defense signal has been well documented (Hayat *et al*., 2010). In addition, different effects of SA on antioxidative enzymes activities could be associated with H_2O_2 metabolism (Hayat *et al*., 2010). Several such signaling molecules as calcium, jasmonic acid, ethylene and salicylic acid have been identified in plants (Jing-Hua *et al*., 2008). It has been reported that exogenous SA application raised the cytoplasmic Ca^{2+} level which is well documented as a messenger in various plants (Bonza *et al*., 2000). The main object of this study was to test the various concentrations of exogenous SA application on cucumber seedlings properties under different water deficit conditions for detecting best dose of SA application for increasing cucumber tolerance to water stress.

Material and methods

Experimental design

This study was carried out as a factorial experiment (3×5) based on complete randomized design with three replications in experimental greenhouse of Ferdowsi University of Mashhad, Iran under controlled conditions. First factor was three water deficit levels (100% FC (I1), 80% FC (I2), and 60% FC (I3)), and second factor was five levels of SA concentrations (0 (S1), 0.25 (S2), 0.5 (S3), 0.75 (S4), and 1 (S5) mM). Seeds of cucumber (Super Dominus cultivar) provided from Khorasan Agricultural Research Station of Iran.

Growth conditions and plant measurement

The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with distilled water prior to applying

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any treatment. Seeds of cucumber (one seed per pot) was sown in 1-2 cm depth in 1-L plastic pots (120 mm ×150 mm) and filled with soil-sand-litter mixture (1:1:1). Irrigation schedule according to water deficit treatments were performed by soil field capacity (FC) calculation. Calculation of amount of the water in FC conditions performed by following equation:

$$\text{Water at the field capacity} = \frac{\text{soil mass at field capacity - over dry soil mass}}{\text{over dry soil mass}} \times 100$$

Each pot daily weighted and adds required water to reach to determined percentage of FC. Plants were grown in 18-25°C, 85 $mmol.m^{-2}.s^{-1}$ daily radiations, and 65% relative humidity. When the first leaf was appeared the plants were sprayed with different levels of SA solved into distilled water. Plant measurements were included: leaf area per pot, stomatal conductance ($cm^{-2}.s^{-1}$), leaf and root dry weight per pot and SPAD reading when fourth leaf was fully expend. Collected materials were weighted after drying in an oven at 45°C for 72 h. Stomatal conductance and SPAD measurements performed by leaf prometer SC-1 and SPAD 502 Minolta, respectively.

Statistical analysis

In order to evaluate the treatments impacts on study parameters, analysis of variance (ANOVA) was performed as standard procedure for factorial complete randomized designs. The t-test was used to find significant differences among treatments. The significant differences between treatments were compared by Duncan's multiple range tests at 5% probability level.

Results and discussion

Water deficit

All study parameters except root dry weight was statistically influenced ($P < 0.05$) by different water deficit levels (Tab. 1). The highest and lowest values of leaf area (41.9 and 24.8 cm^2 pot^{-1}) and leaf dry weight (0.23 and 0.16 gr pot^{-1}) obtained from control and maximum levels of water deficit (60% FC), respectively (Tab. 1). Similar results were observed when water deficit impacts were evaluated on stomatal conductance, increasing of water stress levels showed sharply decline in this parameter. However, the highest SPAD reading (47) reached in extreme levels of water stress (Tab. 1).

Leaf expansion is extremely influenced by water status of plants especially in sensitive plants such as cucumber owing to the fact that water plays vital role in leaf developments of plants (Jelonek *et al*., 2009). In addition, there was direct correlation between leaf weight and leaf area (Liu and Stutzel, 2004) therefore decreasing of leaf area was main reason of decline of leaf dry weight. Stomatal conductance relationship with water situation of plants is well documented (Akinci and Losel, 2010). SPAD reading shows plant chlorophyll density and nitrogen status in leaves (Nakano *et al*., 2010), increasing of chlorophyll density by decreasing of leaf area was expected in higher levels of water stress.

SA concentration

Different SA concentrations showed significant impact ($P < 0.05$) on all studied parameters except for SPAD reading (Tab. 2). The highest values of leaf area (45.3 cm^2 pot^{-1}), leaf (0.27 gr pot^{-1}) and root (0.26 gr pot^{-1}) dry weights obtained in maximum concentration of SA (1 mM) (Tab. 2). Generally, increasing the concentration of SA significantly increased the values of leaf area, leaf and root dry weights. On the other hand, utmost value of stomatal conductance (57.5 cm^{-2} s^{-1}) gained in control treatment (Tab. 2).

Previous studies have offered considerable evidence concerning the stimulation of SA to water stress tolerance (Gunes *et al*., 2007). Hmada and Hakimi (2001) have shown that the treatment of wheat plants with 100 ppm SA through seed soaking was able to alleviate inhibitory effect of drought and stimulate growth by enhancing photosynthetic rate and reducing dark respiration.

Interactive effects of water deficit and SA concentration

The results showed that maximum leaf area value (55 cm^2 per pot) and the highest interaction between SA concentrations and water deficit levels was obtained under 0.75 mM SA application and second water deficit level (80%) FC (Tab. 3). Moreover, leaf dry weight dramatically increased in severe water deficit level by increasing in SA concentration in particular in 1 mM SA concentration (30 gr per pot) (Tab. 3). Different SA concentrations did not show direct impact on SPAD reading values in under various water stress levels but values of this parameter gradually increased in severe water stress levels (Tab. 3).

Salicylic acid is well known obviously taking place signaling molecule that plays a vital role in establishing and signaling a defense response against abiotic stress (Hayat *et al*., 2010). Exogenously application of SA in suitable

Tab. 1. Effects of different water deficit levels on leaf area, stomatal conductance, leaf and root dry weight, and SPAD reading of cucumber

Water deficit level	Leaf area (cm^2.pot)	Leaf dry weight (gr per pot)	Root dry weight (gr per pot)	Stomatal conductance ($cm^{-2}.s^{-1}$)	SPAD reading
I1	41.9^a	0.23^a	0.24^{ns}	58.2^a	41^b
I2	41.6^a	0.22^a	0.22^{ns}	54.0^a	40^b
I3	24.8^b	0.16^b	0.20^{ns}	25.6^b	47^a

I1: 100% FC, I2: 80% FC, and I3: 60% FC

Tab. 2. Effects of various SA concentrations on leaf area, stomatal conductance, leaf and root dry weight, and SPAD reading of cucumber

SA concentration	Leaf area ($cm^2.pot^{-1}$)	Leaf dry weight ($gr.pot^{-1}$)	Root dry weight ($gr.pot^{-1}$)	Stomatal conductance ($cm^{-2}.s^{-1}$)	SPAD reading
S1	27.4^{b}	0.16^{b}	0.14^{b}	57.5^{a}	41^{a}
S2	36.6^{a}	0.17^{ab}	0.25^{a}	54.5^{a}	43^{a}
S3	35.6^{a}	0.20^{ab}	0.18^{ab}	46.8^{ab}	40^{a}
S4	37.6^{a}	0.25^{a}	0.25^{a}	42.3^{ab}	42^{a}
S5	45.3^{a}	0.27^{a}	0.26^{a}	28^{b}	45^{a}

S1: 0 mM, S2: 0.25, S3: 0.5 mM, S4: 0.75 mM, and S5: 1 mM

Tab. 3. Interactive effects of different water deficit levels and various SA concentrations on leaf area, leaf and root dry weight

SA concentration	Water deficit level														
	Leaf area ($cm^2.pot^{-1}$)			Ratio		Leaf dry weight ($gr.pot^{-1}$)			Ratio		SPAD reading			Ratio	
	I1	I2	I3	I2/I1	I3/I1	I1	I2	I3	I2/I1	I3/I1	I1	I2	I3	I2/I1	I3/I1
S1	38	35	14	0.9	0.4	0.19	0.21	0.10	1.1	0.5	35	39	49	1.1	1.3
S2	52	25	21	0.5	0.8	0.16	0.17	0.23	1.1	1.4	41	41	47	1.0	1.1
S3	44	51	36	1.2	0.7	0.20	0.19	0.24	1.0	1.3	40	39	43	1.0	1.1
S4	25	51	45	2.0	0.9	0.25	0.23	0.16	0.9	0.7	42	42	44	1.0	1.0
S5	52	55	26	1.1	0.5	0.37	0.27	0.30	0.7	1.1	47	38	51	0.8	1.3

I1: 100% FC, I2: 80% FC, I3: 60% FC; S1: 0 mM, S2: 0.25, S3: 0.5 mM, S4: 0.75 mM, and S5: 1 mM

concentrations may enhance the efficiency of antioxidant system in plants (Hayat *et al*., 2010). Khan *et al*. (2003) reported an increase in transpiration rate and stomatal conductance in response to foliar application of SA in corn and soybean under stress conditions. Foliar application of SA also proved to increasing the pigment contents and chlorophyll content in *Brassica napus* (Ghai *et al*., 2002). In conclusion, application of SA in suitable concentrations (0.75 mM) could good opportunity to decrease of water stress injures on cucumber seedlings.

Conclusions

It is well documented that SA potentially generates a wide collection of metabolic responses in plant also affects the photosynthetic parameters and plant water relations. The protective effect of SA under abiotic stress such as water deficit is generally coupled with photosynthetic performance (Szepesi *et al*., 2009). In general, increasing the water deficit levels directly decline leaf area, dry weight and stomatal conductance. On the other hand, application of SA gradually mitigated the negative effects of water deficit stress especially, on leaf area expansion. Moderate levels of SA application (0.75 mM) showed highest performance under higher levels of water deficit. To sum up, the results of recent study conformed recovery impacts of SA application under water deficit conditions on cucumber seedlings.

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