Physiological Response of Sweet Wormwood to Salt Stress under Salicylic Acid Application and Non Application Conditions

Kourosh Eskandari Zanjani[1*], Amir Hossein Shirani Rad[1], Zahra Bitarafan[2], Amin Moradi Aghdam[1], Tofigh Taherkhani[1], Pezhman Khalili[1]

[1]Department of Agronomy, Takestan Branch, Islamic Azad University, Takestan, Iran
[2]Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran
* Corresponding Author: K.eskandarizanjani@tiau.ac.ir, Tel.: 00989122416401

Abstract: Soil salinity is one of the major environmental stresses affecting plant growth and productivity. To assess the effect of salt stress on physiological traits of sweet wormwood medicinal plant (Artemisia annua L.) under salicylic acid application and non application conditions a field study was conducted in Zanjan, Iran during 2010-2011 crop year in a four- replicated- factorial design laid out in randomized complete block with four salinity levels (0 (control), 4, 8, and 12 ds. m^{-1} NaCl) and two salicylic acid levels (salicylic acid non application (control) and salicylic acid application (0.5 mM solution). Chlorophyll a content, chlorophyll b content, leaf electrical conductivity, stomatal resistance, leaf relative water content, and canopy temperature difference were determined. Results revealed that application of salicylic acid in both stress and non stress conditions increased the chlorophyll a and b content and also leaf relative water content, although leaf electrical conductivity, stomatal resistance and canopy temperature difference decreased by salicylic acid application in both stress and non stress conditions.
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1. Introduction

Sweet wormwood (Artemisia annua L.) belonging to Asteraceae family is an aromatic annual plant which uses as a medicinal plant due to several types of essential oils and alkaloid and glycoside compounds (Challa and Ravindra, 1998). The leaves of A. annua are a rich source of artemisinin, a sesquiterpene lactone used as the raw material for the production of semi-synthetic derivatives that are more stable, bioavailable, and effective against chloroquine-resistant strains of Plasmodium falciparum (Klayman, 1985; Luo and Shen, 1987; Balint, 2001; Marchese et al., 2001; Enserink, 2005; Marchese et al., 2005). Besides its antimalarial activity, artemisinin has proved effective against different types of cancer, parasitic diseases like Schistosomiasis, viral hepatitis B and some animal diseases are also known to be treated by artemisinin (Efferth 2009; Ferriera and Gonzalez, 2008).

It has been demonstrated that plant growth behavior and production of artemisinin in plant parts of A. annua are affected not only by genotype but also by environmental factors (Ferreira et al. 1995). Irradiation (Wang et al. 2007), salinity stress (Qureshi et al., 2005; Qian et al., 2007), chilling stress (Feng et al., 2009) and Dimethyl sulfoxide elicitation (Mannan et al., 2010) have been reported to affect plant growth and artemisinin production. In addition, phytohormones involved in the plant defense response (e.g., abscisic acid (Jing et al., 2009) and salicylic acid (Pu et al., 2009) have also been shown to play important roles in artemisinin accumulation.

High salinity in soil or irrigation water is a common environmental problem affecting plant growth and productivity by provoking osmotic stress and ion toxicity together with induction of oxidative stress. Salinity induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes thus making it difficult for roots to extract water from their surrounding media (Sairam et al., 2002). The effect of high salinity on plant can be observed at the whole plant level in terms of plant death and/or decrease in productivity (Parida and Das, 2004). Salinity decreases germination (Gehlot et al., 2005; Sharma et al., 2004), dry matter accumulation, the rate of net CO_2 assimilation, relative growth, leaf cell expansion and ultimate leaf growth (Cramer et al., 2001; Saqib et al., 2004; Mansour et al., 2005).

Salicylic acid acts as a potential plant growth regulator and plays an important role in regulating a number of plant physiological and biochemical processes. Salicylic acid is a phytohormone witch roles in signal transduction of a wide range of defense responses including the biosynthesis of some secondary metabolites (Hayat et al., 2010; Pieterse and van Loon, 1999). A survey of literature indicates that salicylic acid could affect antioxidant enzyme activities and then cause a moderate increase in the content of reactive oxygen species such as hydrogen peroxide (H_2O_2) (Ali et al., 2006; Chen et al., 1993; Harfouche et al., 2008; Mahdavian et al., 2007) which acts as a second messenger in regulating plant defense responses (Dempsey and Klessig, 1994; Hayat et al., 2010; Jaspers and Kangasjarvi, 2010). Gao Bin et al. (2009) provide evidence that salicylic acid can activate A. annua growth and artemisinin production. Salicylic acid is a phenolic phytohormone involved in regulating plant growth, development, photosynthesis, transpiration, ion uptake and transport, and defenses against abiotic stresses (Gao-Bin et al. 2009). Aftab et al (2011) reported salicylic acid, when applied at 1.00 mM, provided considerable protection against salt stress imposed by adding 50, 100, or 200 mM NaCl to soil.

Considering the aforementioned points, this study conducted with the objective to determine the effect of salicylic acid application on physiological traits of sweet wormwood (A. annua L.) under salt stress condition.

2. Materials and methods

2.1. Experimental site and design

To assess the effect of salt stress on physiological traits of sweet wormwood medicinal plant (*Artemisia annua*) under salicylic acid application and non-application conditions a factorial experiment based on randomized complete block design in four replications was carried out at the experimental farm located in Zanjan, Iran (36°40′ N, 48°29′ E; 1638 m Elevation) during 2010- 2011 crop year. Treatments included four salinity levels (0 (control), 4, 8, and 12 ds m^{-1} NaCl) and two salicylic acid levels (salicylic acid non application (control) and salicylic acid application (0.5 mM solution)).

Seeds were planted in nursery on 1×1 m^2 beds after sterilizing with sodium hypo chloride for 5 min and ethanol (96%) for 30 sec and rinsing with distilled water for several times to remove excess of chemicals. Two month seedlings were transferred to 30 cm in diameter× 50 cm in height plastic pots (20 kg soil per pot). 50 days after transferring, plants were treated with different salinity and salicylic acid levels. Salinity levels in high salinity treatments were increased gradually to prevent sudden stress and plant death.

2.2. Measurements of traits

Chlorophyll a content, chlorophyll b content, leaf electrical conductivity, stomatal resistance, leaf relative water content, and canopy temperature difference were determined.

Canopy temperature was measured using an infrared thermometer (Pyropen D, Calex Electronics Ltd.) in the morning.

At sampling stage young, mature and well developed leaves were used to measure the leaf relative water content. These leaves transferred to laboratory immediately after separation from plant, cleaned and their wet weight measured by 0.001 accuracy scale. The samples put in distilled water for 24 h under no light condition in laboratory. Their saturated weight determined. The leaves were put in electrical oven for 48 h in 80°C and their dry weight measured. The leaf relative water content determined according to following equation (Turner and Jones 1980):

Leaf Relative Water Content (RWC) =

$$\frac{leaf\ wet\ weight - leaf\ dry\ weight}{leaf\ saturated weight - leaf\ dry\ weight} \times 100$$

Stomatal resistance was determined with an AP_4 Prometer (Delta-T Devices, UK).

7 days after treatment of plants, leaves of 3 plants in each treatment were used to measure leaf electrical conductivity. In laboratory, leaves were cut to 1-cm^2 segments. Segments of each sample were put in one tube containing 10 ml distilled water and tubes were incubated at 25°C on a rotary shaker (100 rpm) for 24 h. Electrical conductivity of bathing solution (EC1) was measured using Micro EC Meter after 24 h. Then each tube autoclaved at 120°C for 20 min to release all electrolytes, cooled to 25°C and the final electrical conductivity (EC2) was measured. Electrolyte leakage rate was determined as: $EL = \frac{EC1}{EC2} \times 100$. The hurt rate of cell membrane permeability was calculated using following equation (Bai Wen-Bo et al., 2008):

HR= (ELT- ELCK) × 100

Where HR is the hurt rate (%) and ELT and ELCK are the electrolyte leakage of the treatment and control, respectively.

To measure chlorophyll a and b content, chlorophyll extraction was performed by putting leaf segments in 80% aqueous acetone at 4°C in darkness during the night. The extract was centrifuged for 5 min at 10000× g and absorption rate of supernatant fraction in 645, 663 and 480 nm wavelengths were determined using Spectrophotometer (Arnon, 1949).

2.3. Statistics

Statistical analyses of data were performed with a personal computer using the SAS software. A factorial analysis of variance (ANOVA) was performed for all parameters. In addition the Duncan's Multiple Range Test (DMRT) (P = 0.05) was used to conduct mean comparison.

3. Results and discussion

3.1. Chlorophyll a content

The simple effects of salicylic acid and salinity and the interaction effect of them on chlorophyll a content were all significant at $P= 0.01$ (Table 1). Application of salicylic acid on average 0.927 µm g(fw)$^{-1}$ showed a significant preference in comparison to non application of salicylic acid on average 0.857 µm g(fw)$^{-1}$ in chlorophyll a content more production. Also chlorophyll a content decreased by increasing salinity level from 0 to 12 ds m^{-1} as the lowest rate of chlorophyll a content on average 0.687 µm g(fw)$^{-1}$ obtained in salinity level of 12 ds m^{-1} (Table 2). Study of the interaction effect of treatments revealed that the highest chlorophyll a content on average 1.111 µm g(fw)$^{-1}$ and the lowest chlorophyll a content on average 0.627 µm g(fw)$^{-1}$ obtained in salinity level of 0 ds m^{-1} under application of salicylic acid condition and salinity level of 12 ds m^{-1} under non application of salicylic acid condition, respectively. Application of salicylic acid in both stress and non stress conditions increased the chlorophyll a content (Table 3).

3.2. Chlorophyll a content

The simple effects of treatments and their interaction effect on chlorophyll b content were all significant at $P= 0.01$ (Table 1). Application of salicylic acid on average 0.919 µm g(fw)$^{-1}$ showed a significant preference in comparison to non application of salicylic acid on average 0.799 µm g(fw)$^{-1}$ in chlorophyll b content production . Also chlorophyll b content decreased by increasing salinity level as the lowest rate of chlorophyll b content on average 0.595 µm g(fw)$^{-1}$ obtained in salinity level of 12 ds m^{-1} (Table 2). Study of the interaction effect of treatments showed that the highest chlorophyll b content on average 1.212 µm g(fw)$^{-1}$ and the lowest chlorophyll b content on average 0.544 µm g(fw)$^{-1}$ obtained in salinity level of 0 ds m^{-1} under application of salicylic acid condition and salinity level of 12 ds m^{-1} under non application of salicylic acid condition, respectively. Application of salicylic acid in both stress and non stress conditions increased the chlorophyll b content (Table 3). Aftab et al. (2010) also showed application of salicylic acid positively improved chlorophyll and carotenoid contents in sweet wormwood. Aftab et al. (2011) reported salinity reduced the values of photosynthetic attributes and total chlorophyll content and inhibited the activities of nitrate reductase and carbonic anhydrase in sweet wormwood.

3.3. Leaf electrical conductivity (EC)

The simple effects of treatments and interaction effect of them on leaf electrical conductivity were all significant at P= 0.01 (Table 1). Non application of salicylic acid on average 2003.25 µs cm-1 had higher leaf electrical conductivity in comparison to application of salicylic acid on average 1752.00 µs cm-1. Also leaf electrical conductivity increased by increasing salinity level from 0 to 12 ds m-1 as the highest rate of leaf electrical conductivity on average 2439.50 µs cm-1 obtained in salinity level of 12 ds m-1 (Table 2). Study of the interaction effect of treatments revealed that the highest leaf electrical conductivity on average 2597 µs cm-1 and the lowest leaf electrical conductivity on average 1208 µs cm-1 obtained in salinity level of 12 ds m-1 under non application of salicylic acid condition and salinity level of 0 ds m-1 under application of salicylic acid condition, respectively. Application of salicylic acid in both stress and non stress conditions decreased the leaf electrical conductivity (Table 3). Aftab et al. (2011) reported salt stress significantly increased electrolyte leakage and proline content.

3.4. Stomatal resistance

The simple effects of salicylic acid and salinity and their interaction effect on stomatal resistance were all significant at P=0.01 (Table 1). Non application of salicylic acid on average 3.732 s cm^{-1} showed a higher stomatal resistance in comparison to application of salicylic acid on average 2.995 s cm^{-1}. Stomatal resistance increased by increasing salinity level from 0 to 12 ds m^{-1} as the highest rate of stomatal resistance on average 5.46 s cm^{-1} obtained in salinity level of 12 ds m^{-1} (Table 2). Study of the interaction effect of salicylic acid and salinity revealed that the highest stomatal resistance on average 5.94 s cm^{-1} and the lowest stomatal resistance on average 1.75 s cm^{-1} obtained in salinity level of 12 ds.m^{-1} under non application of salicylic acid condition and salinity level of 0 ds m^{-1} under application of salicylic acid condition, respectively. Application of salicylic acid in both stress and non stress conditions decreased stomatal resistance (Table 3).

3.5. Leaf relative water content (RWC)

The simple effects of treatments and their interaction effect on leaf relative water content were all significant at $P= 0.01$ (Table 1). Application of salicylic acid on average 87.75% showed a significant preference in comparison to non application of salicylic acid on average 83.72%. Also leaf relative water content decreased by increasing salinity level from 0 to 12 ds m^{-1} as the lowest rate of leaf relative water content on average 76.70% obtained in salinity level of 12 ds m^{-1} (Table 2). Study of the interaction effect of treatments revealed that the highest rates of leaf relative water content obtained in salinity level of 0 ds m^{-1} under both application and non application of salicylic acid conditions on average 94.8% and 93.9%, respectively. The lowest leaf relative water content on average 75.1% obtained in salinity level of 12 ds m^{-1} under non application of salicylic acid condition. Application of salicylic acid in stress condition increased the leaf relative water content (Table 3).

Table 1. Analysis of variance components for assessed traits

S.O.V.	DF	***CaC***	***CbC***	***LEC***	***SR***	***LRWC***	***CT***
Replication	3	*	ns	**	**	**	**
Salicylic acid	1	**	**	**	**	**	**
Salinity	3	**	**	**	**	**	**
Salicylic acid × Salinity	3	**	**	**	**	**	**
Error	21						
Total	31	-	-	-	-	-	-
CV	-	1.5903	2.4209	0.3443	2.2248	0.8147	2.3029

*, ** – significant at 5% and 1% respectively, ns – not significant

Table 2. Simple effects of salicylic acid and salinity on assessed traits

Treatments	Mean					
	CaC (µm g(fw)$^{-1}$)	***CbC*** (µm g(fw)$^{-1}$)	***LEC*** (µs cm^{-1})	***SR*** (s cm^{-1})	***LRWC*** (%)	***CTD*** (°C)
Salicylic acid						
Non application	0.857 b	0.799 b	2003.25 a	3.732 a	83.72 b	-3.45 b
Application	0.927 a	0.919 a	1752.00 b	2.995 b	87.75 a	-2.755 a
Salinity (ds m^{-1})						
0	1.100 a	1/119 a	1261.50 d	1.82 d	94.35 a	-1.815 a
4	0.959 b	0.951 b	1710.50 c	2.50 c	89.25 b	-2.485 b
8	0.821 c	0.771 c	2099.00 b	3.59 b	82.65 c	-3.565 c

Any two means sharing a common letter do not differ significantly from each other at 5% probability.

Table3. Interaction effect of salicylic acid and salinity on assessed traits

Treatments		Mean					
		CaC (µm g(f w)$^{-1}$)	***CbC*** (µm g (f w)$^{-1}$)	***LEC*** (µs cm^{-1})	***SR*** (s cm^{-1})	***LRWC*** (%)	***CTD*** (°C)
Salicylic acid	Salinity						
Non application	0	1.09 b	1.03 b	1315 g	1.89 f	93.9 a	-1.87 b
	4	0.921 d	0.905 d	1879 e	2.98 d	86.2 c	-2.99 d
	8	0.793 f	0.721 f	2222 c	4.12 c	79.7 d	-3.97 f
	12	0.627 h	0.544 h	2597 a	5.94 a	75.1 f	-4.97 h
Application	0	1.11 a	1.212 a	1208 h	1.75 g	94.8 a	-1.76 a
	4	0.998 c	0.997 c	1542 f	2.02 e	92.3 b	-1.98 c
	8	0.849 e	0.822 e	1976 d	3.07 d	85.6 c	-3.16 e
	12	0.747 g	0.646 g	2282 b	4.98 b	78.3 e	-4.12 g

Any two means sharing a common letter do not differ significantly from each other at 5% probability.

Traits in *italic*, ***CaC***, ***CbC***, ***LEC***, ***SR***, ***LRWC***, and ***CTD*** are assigned for chlorophyll a content in µm g(fw)$^{-1}$, chlorophyll b content µm g(fw)$^{-1}$, leaf electrical conductivity in µs cm^{-1}, Stomatal resistance in s cm^{-1}, leaf relative water content in %, and canopy temperature difference in °C, respectively.

3.6. *Canopy temperature difference*

The simple effects of salicylic acid and salinity and their interaction effect on canopy temperature difference were all significant at $P = 0.01$ (Table 1). Non application of salicylic acid on average -3.45°C showed a higher difference in comparison to application of salicylic acid on average -2.755°C. Also canopy temperature difference increased by increasing salinity level from 0 to 12 ds m^{-1} as the highest rate of canopy temperature difference on average -4.545°C obtained in salinity level of 12 ds m^{-1} (Table 2). Study of the interaction effect of treatments showed that the lowest canopy temperature difference on average -1.76°C obtained in salinity level of 0 ds m^{-1} under application of salicylic acid condition. The highest canopy temperature difference on average -4.97°C obtained in salinity level of 12 ds m^{-1} under non application of salicylic acid condition. Application of salicylic acid in both stress and non stress conditions decreased the canopy temperature difference (Table 3).

4. Conclusion

Adjustments in chemical elicitors such as salicylic acid can be used as an effective method to increase *A. annua* growth and powered defense responses. This study provides new findings about the physiological changes in *A. annua* in response to salicylic acid application under salt stress condition. Our results revealed that chlorophyll a and b content and leaf relative water content increased by application of salicylic acid and decreased by increasing salinity level lonely. Although leaf electrical conductivity, stomatal resistance and canopy temperature difference responded differently and decreased by salicylic acid application and increased by increasing salinity level. The highest and lowest chlorophyll a content b content and leaf relative water content obtained in salinity level of 0 ds m^{-1} under application of salicylic acid condition and salinity level of 12 ds m^{-1} under non application of salicylic acid condition, respectively. The highest and the lowest leaf electrical conductivity, stomatal resistance and canopy temperature difference obtained in salinity level of 12 ds m^{-1} under non application of salicylic acid condition and salinity level of 0 ds m^{-1} under application of salicylic acid condition, respectively. Application of salicylic acid in both stress and non stress conditions increased the chlorophyll a content and b content and leaf relative water content, although leaf electrical conductivity, stomatal resistance and canopy temperature difference decreased by salicylic acid application in both stress and non stress conditions.

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