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Morphological traits and yield of Ajowan affected by different irrigation intervals and growth regulators

S. Ghassemi [1] (*), S. Zehtab-Salmasi [1], K. Ghassemi-Golezani [1], S. Alizadeh-Salteh [2]

[1] *Department of Plant Eco-physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.*

[2] *Department of Horticulture, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.*

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(*) **Corresponding author:**
saeid.ghassemi67@gmail.com

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Abstract: Two field experiments were carried out to evaluate the effect of salicylic acid and abscisic acid applications on some morphological traits and yield of ajowan (*Carum copticum* L.) under different watering conditions. Irrigation intervals (irrigation after 70, 100, 130 and 160 mm evaporation from class A pan) were located in main plots and exogenous applications of water (control), salicylic acid (0 and 1 mM) and abscisic acid (0 and 50 micro-molar) were allocated to sub plots. Water stress negatively affected the morphological traits of the Ajowan. Stem diameter, branches per plant, leaves number per plant, biological yield and grain yield per plant of Ajowan were considerably improved by application of salicylic acid and abscisic acid. Salicylic acid treatment was more effective than abscisic acid treatment, in this study, salicylic acid treatment increased the stem diameter and leaves number per plant more than abscisic acid and caused a yield enhancement in Ajowan plants. In general, foliar spray of salicylic acid and abscisic acid could alter morphological traits and yield of Ajowan.

1. Introduction

Medicinal plants have been used for centuries as remedies for diseases and human health, because they contain secondary metabolites of medicinal value. Ajowan (*Carum copticum* L.) is an annual herbaceous plant belonging to the *Umbelliferae* family, which grows in the east of India, Iran, and Egypt, with white flowers and small, brownish seeds. Its fruit has been widely consumed as a food flavoring agent and spice. During the past centuries in the Iranian traditional medicine, several therapeutic effects including anti-vomiting, antiasthma and anti-spasm, is postulated for ajowan fruits (Boskabady *et al.*, 2005).

Water deficit, defined here as an unbalance between soil water availability and evaporative demand which can naturally occur in the field and causes a decrease in carbon assimilation, tissue expansion and actually cell number. Each of these macroscopic processes involves a large number of genes, enzymes, hormones and metabolites (Skirycz and Inze, 2010). Abiotic stresses such as drought cause metabolic changes ranging from synthesis of limited quantities of specialized metabolites to large shifts in primary metabolite composition as well as many other physiological responses. During drought there is a need for osmoticum to accumulate inside the plant cell to retain water and maintain positive turgor pressure (Verslues and Juenger, 2011). It is generally accepted that, at whole plant level, adaptation to water depletion starts with a change in stomatal conductance, leading to reduced net CO_2 assimilation and impaired photosynthesis, which result in shoot growth termination. The result of Nassiri *et al.* (2014) showed that seed yield, water use efficiency and harvest index of ajowan reduced in water stress treatment. Also, Fresh and dry weight of *Ocimum sp.* significantly reduced in water stress conditions (Khalid, 2006). Adaptation to water stress can be highly controlled by plant growth regulators (Popko *et al*., 2010).

Salicylic acid is a phenolic compound capable of enhancing plant growth and yield in some plants (Arfan *et al*., 2007). Salicylic acid can regulate various stress responses and development such as abiotic stress responses, flowering, senescence, thermogenesis and resistance to pathogens (Vicente and Plasencia, 2011). Among abiotic stresses, this growth regulator has been reported to counter low temperature (Tasgin *et al*., 2003), water stress (Abbaspour and Ehsanpour, 2016), salinity stress (El Tayeb, 2005) and high temperature (He *et al*., 2005). Salicylic acid plays diverse physiological roles in most of plants, which include nutrient uptake, stomatal movements, enzyme activities, thermogenesis, ethylene biosynthesis, photosynthesis, flower induction and plant growth (Hayat and Ahmad, 2007). Applications of 1 or 2 mM SA significantly reduced chilling injury and fruit decay of apricot fruit as well as membrane electrolyte leakage and ascorbic acid content. Fruits treated with SA resulted in high total polyphenolic content, antioxidant capacity and carotenoids content (Ezzat *et al*., 2017). Also, application of SA significantly enhanced activity of phenylalanine ammonialyase (PAL) and content of hydrogen peroxide in apricot fruit. Treated fruits showed significantly lower activity of catalase and ascorbate peroxidase but higher activity of superoxide dismutase and peroxidase than those in control fruits (Wang *et al*., 2015). Exogenous application of salicylic acid or its derivates affects diverse plant processes. Since, this growth regulator is heavily involved in crosstalk with other plant hormones; its effect on some of these processes may be indirect (Pieterse *et al*., 2009).

Abscisic acid plays critical roles in numerous biological processes, such as gene transcription, seed dormancy and stomatal closure (Cutler *et al*., 2010). This growth regulator appears to be a major player in mediating the adaptation of the plant to water stress (Pal *et al*., 2011). Abscisic acid enhances drought tolerance in wheat and many of the other plant species (Travaglia *et al*., 2010). That plays an important role in the regulation of abiotic stress resistance in plants and orchestrates complicated signalling pathways involved in the response to reduced water availability as well as in multiple developmental processes (Kim *et al*., 2010).

Based on the aforementioned studies and other researchers, it is acceptable that exogenous application of salicylic acid and abscisic acid can improve abiotic stress tolerance in plants. Thus, this study was designed to investigate the effect of salicylic acid and abscisic acid applications on some morphological traits of Ajowan plants under water stress.

2. Materials and Methods

At the Research Farm of the Faculty of Agriculture, University of Tabriz, Tabriz, Iran, during the growing seasons of 2014 and 2015, two field experiments were carried out to evaluate the effect of salicylic acid and abscisic acid applications on some morphological traits and yield of *Carum copticum* L. under different irrigation treatments. Irrigation intervals [I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan (a cylinder with a diameter of 120.7 cm that has a depth of 25 cm) as normal irrigation and mild, moderate and severe water deficit, respectively] were assigned to main plots and foliar applications of water (control), salicylic acid (0 and 1 milli-molar) and abscisic acid (0 and 50 micro-molar) were allocated to subplots. Average maximum and minimum temperatures and rainfall during the experiment in 2014 and 2015 are shown in Table 1.

Each plot had 6 rows of 4 m length, spaced 25 cm apart. Seeds of this plant were treated with benomyl (Benlat wp 50%; Shanghai Bosman Industrial Co. Ltd, China) at a rate of 2 g kg^{-1} before sowing. Then sown by hand on April 2014 and 2015 at a depth of about 1 cm in a sandy-loam soil. All plots were regularly irrigated after sowing until seedling establishment, and thereafter irrigations were carried out according to the treatments. During plant growth and development, weeds were controlled by hand as required. Salicylic acid and abscisic acid were sprayed on plants at vegetative (once) and reproductive (once) stages.

Table 1 - Averages of maximum and minimum temperatures and rainfall during the work in Tarbiz, Iran, 2014-2015

Month	Temperature (°C)		Rainfall (mm)	
	2014	2015	2014	2015
April	23.7	12.6	50.2	43.2
May	29.5	30.45	10.7	1.5
June	37.1	38.9	18	0.9
July	38.9	40.2	1.3	0
August	34.75	31.1	0	26.4
September	20.65	23.45	84.2	24.1

Leaf number per plant was measured by hand at grain filling stage. Also, at maturity stage, other traits such as plant height (by meter), stem diameter (by digital caliper) and branches per plant (count by hand) were determined. At maturity, 10 plants of the middle part of each plot were harvested and grain yield per plant were determined. Then above ground biomass was oven-dried at 80°C for 48 hours and weighed and subsequently plant biomass was calculated. Measurements for all morphological traits were normally distributed and confirmed through the Kolmogorov-Smirnov test. Randomness was confirmed using the Run Test, and the descriptive statistics were calculated. MSTATC software used to the data analyzed and the means of traits were compared using Duncan multiple range tests at *P≤0.05*.

3. Results and Discussions

Combined analyses of variance showed significant effects of irrigation and growth regulators on stem diameter, branches per plant and leaf number per plant, biomass and grain yield per plant. Plant height was affected by irrigation treatments and plant biomass was significantly influenced by year. The interaction of year × irrigation, year × growth regulator and year × irrigation × growth regulator for plant biomass were significant. Grain yield per plant significantly affected by interaction of irrigation × growth regulator (Table 2).

Plant height of Ajowan decreased with increasing water stress. There were no significant differences between hormonal treated and control plants (Fig. 1). Stem diameter decreased with increasing irriga-

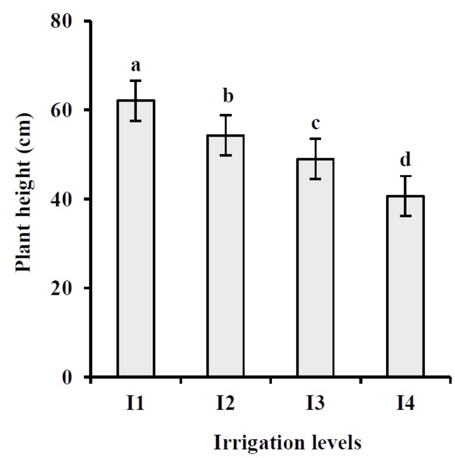

Fig. 1 - Means of plant height of ajowan for different irrigation levels. Different letters in each column indicate significant difference at p≤0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Table 2 - Combined analysis of variance of morphological traits of Ajowan under different irrigation and growth regulators in Tarbiz, Iran, 2014-2015

Source	df	Mean square					
		Plant height	Stem diameter	Branches per plant	Leaf number per plant	Plant biomass	Grain yield per plant
Year (Y)	1	0.056 NS	0.001 NS	0.68 NS	74.01 NS	65.532 **	10.178 ns
Repeat	4	213.63	0.001	7.94	233.44	24.849	5.580
Irrigation (I)	3	1465.38 **	0.169 **	47.90 **	5662.23 **	709.482 **	294.030 **
Y×I	3	0.05 NS	0.001 NS	1.71 NS	24.82 NS	15.325 *	2.631 ns
Error	12	48.69	0.004	1.35	190.24	3.320	2.420
Growth regulator (G)	2	0.72 NS	0.05 **	6.12 *	1762.16 **	80.355 **	18.847 **
Y×G	2	0.05 NS	0.001 NS	0.10 NS	73.39 ns	5.137 **	0.296 ns
I×G	6	72.50 NS	0.001 NS	2.40 NS	90.44 ns	16.430 **	3.944 **
Y×I×G	6	0.05 NS	0.001 NS	1.07 NS	24.93 ns	2.724 *	0.316 ns
Error	32	54.43	0.002	1.25	67.27	0.834	0.498
CV (%)		14.32	9.86	10.44	16.81	8.53	12.03

NS,*,**= No significant and significant at P≤0.05 and P≤0.01, respectively.

tion intervals. Hormonal application, especially treatment with salicylic acid significantly increased this trait (Fig. 2A and B). With increasing water deficit branches per plant of ajowan decreased. However, difference between I_2 and I_3 was not significant. Salicylic acid treatment increased this trait but, there was no difference between salicylic acid and abscisic acid (Fig. 2C and D). Similarly, leaf number per plant decreased as water deficit increased. Salicylic acid treatment significantly increased this trait. However, there was no significant differences between abscisic acid treated and control plants (Fig. 2E and 2F).

In both years plant biomass of Ajowan decreased with decreasing water supply. Salicylic acid application enhanced this trait under I_1 and I_2 in 2014 and under I_1 in 2015, but abscisic acid treatment only increased plant biomass under I_1 in both years. Salicylic acid was advantage that of abscisic acid on this trait (Fig. 3).

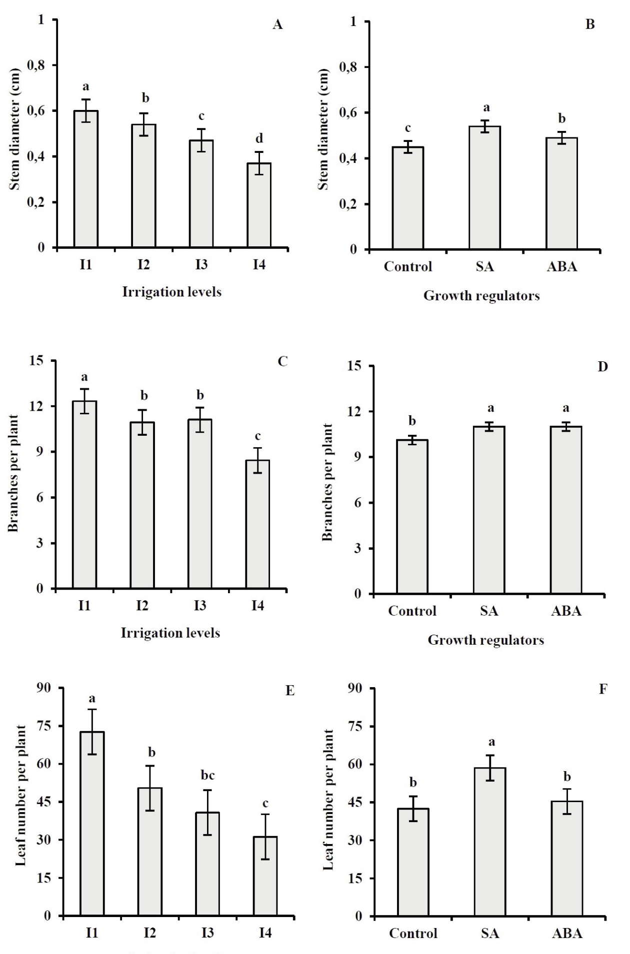

Fig. 2 - Means of stem diameter (A and B), branches per plant (C and D) and leaf number per plant (E and F) of ajowan for different irrigation and growth regulators. Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid; ABA= Abscisic acid.

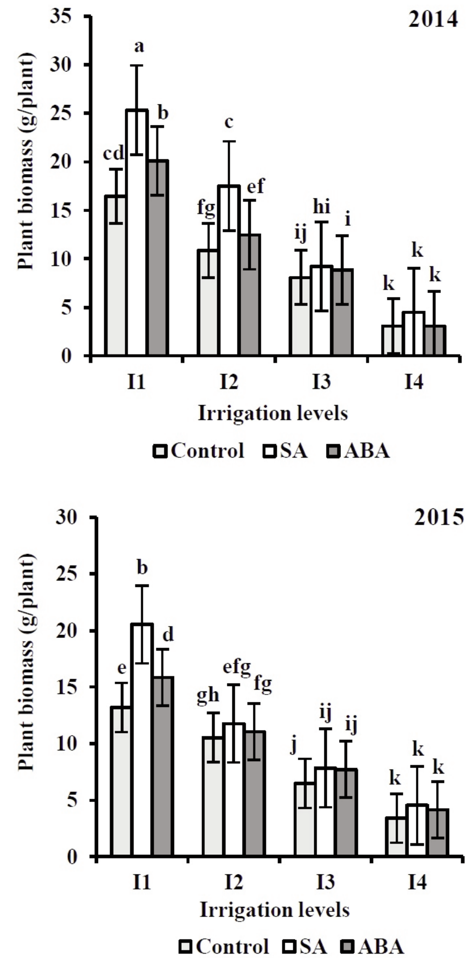

Fig. 3 - Means of plant biomass of Ajowan for year × irrigation × growth regulator. Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test). I1, I2, I3, I4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid - ABA= Abscisic acid.

With increasing water deficit, grain yield of salicylic acid treated plants significantly decreased, compared with control plants. Exogenous application of salicylic acid under I_1 and I_2 and also abscisic acid application under I_2 treatment improved grain yield of ajowan plants. However, under I_3 and I_4 irrigation treatments there was no significant difference between hormonal treated and non-treated plants (Fig. 4).

Reduction in plant height due to water stress (Fig. 1) is related with the competition of plants for nutrients and water availability (Ghassemi-Golezani *et al.*, 2010). Water stress during vegetative stages has the greatest impact on biomass and plant height (Ghassemi-Golezani *et al.*, 2008). It has been confirmed by many researchers that water stress lead to growth reduction, which was reflected in leaf area, plant height, dry mass, and other growth functions (Fischer *et al*., 1980; Kriedemann *et al*., 1981). The impact of water stress on plant growth can be explained as a method of adaptation to the conditions of water shortage to limit the rate of transpiration (Lu and Neumann, 1998), in order to maintain the water supply in the soil around plant roots to increases the chance of survival of the plant (Passioura, 2002). The mechanism, by which plant height is reduced under water stress, is through the reduction of cell elongation, which leads to the reduction of cell size and therefore the reduction of plant height (Schuppler *et al*., 1998).

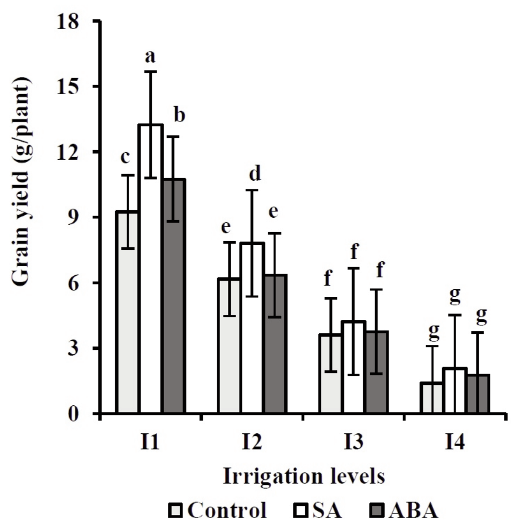

Fig. 4 - Mean grain yield of ajowan for interaction of irrigation × growth regulator. Different letters indicate significant difference at $p \leq 0.05$ (Duncan test). I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively. SA= Salicylic acid, ABA= Abscisic acid.

A reduction in leaf turgor and photosynthesis under water stress condition suppresses cell expansion and growth, leading to the diminution of stem diameter (Fig. 2A) (Anjum *et al*., 2011). The decrease in growth parameters under drought stress could be considered as an avoidance mechanism which minimizes water losses (Rodriguez *et al*., 2005). Stem diameter fluctuates daily because of transpiration-induced tension changes in the stem sap (Irvine and Grace, 1997; Perämäki *et al*., 2001). Salicylic acid application promotes cell division and enlargement (Hayat *et al*., 2005). It has been reported that salicylic acid significantly enhances the average growth speed of tomato stems (Stevens and Senaratna, 2006), which is in line with the results of the present study. Salicylic acid was reported to increase cytokinins in corn and these hormones increased the stem diameter (Shakirova *et al*., 2003). Foliar spray of abscisic acid is favouring vegetative growth of plants as shown for ajowan (Fig. 2B) and soybean (Travaglia *et al*., 2009). Exogenous application of abscisic acid was able to increase plant adaptive response to various environmental conditions (Abraham *et al*., 2008).

Number of branches and leaves per plant decreased under drought condition (Fig. 2C and 2E). This might be related with the suppression of cell expansion and cell growth due to the low turgor pressure. Reduced number of leaves by moisture stress is in line with the finding of Stolf-Moreira *et al*. (2010) who found that water stress affected number of leaves for soybean. This is because water stress leads to decreased rate of leaf initiation and reduction in leaf area of already formed leaves. This can be related with lower photosynthetic activity in the affected leaves. The overall effect is a decrease in the rate of new leaf initiation and increase in leaf shedding thereby resulting to reduction in number of green leaves per plant (Yunusa *et al*., 2014).

Salicylic acid stimulatory effect on growth estimated characteristics could be related with the effect of this growth regulator on the endogenous phytohormones such as growth promoters i.e. cytokinins, gibberellins and auxins (Waffaa *et al*., 1996; Shehata *et al*., 2000). Application of these growth regulators increased number of branches per plant (Fig. 2D) and leaves (Fig. 2F), which could lead to increment of number of flowers and subsequently seed yield. In agreement with these results Fathy *et al*. (2003) on eggplant and Gharib (2007) on basil and marjoram they mentioned that salicylic acid increased plant height, number of branches and leaves per plant and dry mass as well, respectively. Abscisic acid by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance, plays a critical role in regulating plant water status through guard cells and growth as well as (Luan, 2002; Zhu, 2002) which might be the main reason for increased branches per plant. Treatment with abscisic acid in *Sesamum indicum* L. increased number of branches to a large extent (Abraham *et al*., 2008).

Plant biomass was reduced under water stress (Fig. 3) due to leaf senescence and decline in the cell enlargement resulting from reduced turgor pressure (Shao *et al*., 2008). Severe water stress may result in arrest of photosynthesis and disturbance of metabolism (Liang *et al*., 2006). Water stress inhibits cell enlargement and that reduces various biochemical and physiological processes (Shao *et al*., 2007).

Drought stress leading to stomata closure and reduction in photosynthesis rate and leaf growth (Ozturk, 1999), which ultimately decreases plant biomass. This reduction in plant biomass resulted in decreasing the plant height (Fig. 1), stem diameter, branches per plant and leaf number per plant (Fig. 2) and consequently grain yield per plant (Fig. 4).

Salicylic acid improved the production of plant biomass (Fig. 3) by increasing the stem diameter, branches per plant and leaf number per plant (Fig. 2). Salicylic acid influences a wide variety of plant processes, including stomatal regulation, chlorophyll content and photosynthesis (Yildirim *et al*., 2008). Also, by increasing ribulose 1,5-bisphosphate (Rubp) content under drought condition, protecting the photosynthetic machinery from reactive oxygen species produced during drought stress (Shehata *et al*., 2001) and maintaining LAI and photosynthetic activity under stress (Bayat and Sepehri, 2012) increased plant biomass. Abscisic acid plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Zhu, 2002), which might be resulted in increasing dry mass under drought stress (Fig. 3).

Reduction of grain yield under water stress (Fig. 4) has been attributed to reduced plant height (Fig. 1), stem diameter, branches per plant, leaf number per plant (Fig. 2) and plant biomass (Fig. 3). Water stress severely limits growth and yield of plants by reducing chlorophyll content of leaves, photochemical efficiency of photosystem II (Ghassemi-Golezani and Lotfi, 2012), photosynthesis (Munns *et al*., 2006) and ground green cover (Ghassemi-Golezani and Ghassemi, 2013). Water stress during vegetative stages largely reduces plant height and biomass, while during reproductive stages it has the greatest negative impact on grain yield (Ghassemi-Golezani *et al*., 2008).

Grain yield was improved by growth regulators (Fig. 4). The increase in yield might be due to increased sink size mainly number and mass of grains. This could be related to increased photosynthetic efficiency by stabilization of chlorophyll and higher production and translocation of organic material from source to sink. Salicylic acid stimulates physiological processes that were reflected on improving vegetative growth (Fig. 2, 3) followed by active translocation of the photosynthesis products from source to sink. Abscisic acid is also able to activate metabolism of carbohydrates temporally stored in the plant stem (Yang *et al*., 2003; Travaglia *et al*., 2010).

4. Conclusions

Water stress negatively affected the morphological traits and yield of the Ajowan. Stem diameter, branches per plant, leaf number per plant, individual plant biomass and grain yield were considerably improved by application of salicylic acid and abscisic acid. Salicylic acid treatment had more positive effects on plants, compared with abscisic acid treatment. Foliar spray of abscisic acid and especially salicylic acid could, therefore, alter morphology and yield of Ajowan.

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