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Effects of Salicylic acid and Jasmonic acid on Hill Reaction and Photosynthetic Pigment (*Dracocephalum Moldavica* L.) in Different Levels of Drought Stress

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ABSTRACT

Objective: In order to study the effect of drought stress, salicylic acid (SA) and jasmonic acid (JA) foliar application on Hill reaction and photosynthetic pigments. **Methods:** Experiment was done as factorial on the basis of completely randomized design in 4 replications. Irrigation in 3 levels (FC, $\frac{2}{3}$ FC and $\frac{1}{3}$ FC), salicylic acid in 2 levels (0 and 0.5 mM) and jasmonic acid in 2 levels (0 and 50 μM). **Results:** The results indicated that, the effects drought stress, salicylic acid and jasmonic acid on Hill reaction, chlorophyll a, chlorophyll b, chlorophyll a+b, β-carotene and Xanthophyll were significant. Drough stress increased the content of β-carotene (1.83 $\frac{\mu g}{mL}$) and Xanthophyll (2.60 $\frac{\mu g}{mL}$). The 50 (μM) foliar application of jasmonate and salicylic acid under normal irrigation condition gained to maximum amounts of Hill reaction, chlorophyll a, chlorophyll b, chlorophyll a+b, β-carotene and xanthophyll. According to the results, exogenous salicylic acid and jasmonic acid application can improve drought tolerance in moldavian dragonhead (*Dracocephalum moldavica* L.).

1.INTRODUCTION

moldavian dragonhead (*Dracocephalum moldavica* L.) is an annual aromatic resistant plant with green leaves and branches belonging to the *Lamiaceae* family. The origin of the plant is reported to be south of Siberia and Himalayan hillsides. Its compounds are sedative and used as appetizer. Its extract has antibacterial effects and is used as a carminative, for stomachache and also in food and beverage industry, cosmetics and hygienic industry (Said –Al Ahl *at al.,* 2010). Most parts of Iran are located in regions where annual precipitation does not meet the necessary water for agricultural purposes. Thus, drought stress is unavoidable and to achieve favorable crop performance, water shortage should be compensated for by irrigation. Instability of ecological conditions such as water supply and genetic diversity of the plant species make them react to the changes in different ways. Water supply is one of the most important factors that can influence plant's structure and its performance (Sunka *et al*., 2003).

Drought stress is one of the major abiotic stresses in agriculture worldwide. The effect of water stress on plant growth usually declines, changes in carbon and nitrogen metabolism in plants is observed. Plants respond to stress with time and space at different levels of plant organization (Yordav *et al.,* 2003). Chlorophyll content is one of the major factors affecting photosynthetic capacity. Reduction or nochange in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration .Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues (Ganj Arjenaki *et al.,* 2012). Research results showed that the different plants, the chlorophyll content decreased under severe drought stress. Lack of water can destroy

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chlorophyll and make it stop (Lessani & Mojtahedi, 2002). In addition. Other researchers, the damage to the photosynthetic pigments as a result of water shortages have been reported (Ganj Arjenaki *et al.,* 2012). Production of reactive oxygen species such as O_2^- and H_2O_2 organic peroxides stress conditions, membrane lipid peroxidation and the resulting chlorophyll is destroyed (Foyer *et al.,* 1994). Also, the lower leaves are yellow-green chlorophyll content increases. It seems that this mechanism could photosynthetic system to protect against stress (Lawlor & cornic, 2002).

For the first time, the plant material was extracted from *Jasminum grandiflorum* that was called Jasmonic acid (JA) methyl ester. So, as a new hormone (Abdala *et al.,* 2003). Plant hormones play crucial role in regulating plant growth responses to stress. JA and its methyl ester, many biological effects. Such as, the aging and falling leaves, stomatal closure, beta-carotene synthesis, ethylene synthesis and inhibition of root growth (Yu *et al.,* 2000). Jasmonate is also a signal molecule in so-called induced systemic resistance (ISR), wound response (also caused by insect feeding) and elicitation process (Van Wees *et al.* 2000). It is known that JA and its derivative forms, such as methyl jasmonate, represent important plant signal transduction molecules involved in the response to pathogen attack, and their exogenous addition to plant cell culture or intact plants stimulates biosynthesis of a wide range of secondary metabolites (Kuzmaa *et al.* 2009). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature and also an important signaling molecule hich regulates physiological processes in plants such as growth, photosynthesis and some other metabolic processes (Khan *et al.* 2012). Several studies support a major role of SA in modulating the plant response to various abiotic stresses. In addition, it has been found that plants treated with SA generally exhibited better resistance to drought stress (Kadioglu *et al*,. 2011). It is a well-found fact that SA potentially generates a wide array of metabolic responses in plants and also affects plant water relations (Hayat *et al.* 2010). Treatment of drought stressed plants with exogenous SA was found to be effective in modulating both enzymatic and non-enzymatic components of antioxidant defense system (Kadioglu *et al.* 2011). Therefore, the objective of our study was to observe the beneficial role of SA and JA in enhancing the content chlorophyll (Chl), β-carotene and Xanthophyll, also, Hill reaction rate under drought stress condition in moldavian dragonhead seedlings.

2. MATERIALS AND METHODS

Due to study the effect of drought stress, salicylic acid and jasmonic acid foliar application on some photosynthetic pigments and Hill reaction rate, an experiment was done as factorial in the basis of completely randomized design in 4 replication. Irrigation in 3 levels (FC, $\frac{2}{3}$ FC and $\frac{1}{3}$ FC), salicylic acid in 2 levels (0 and 0.5 mM) and jasmonic acid in 2 levels (0 and 50 μM). The extraction and estimation of chlorophyll a, chlorophyll b, β-carotene and Xanthophyll was done according to Jensen (1978). Using extracts of acetone produced by the method Lichtenther (1987) and adding petroleum ether (20 mL) and salt water (3%) (10 ml) phases make. Stage top after adding diethyl ether and salt water to measure chlorophyllα and Xanthophyll used. phased down after adding KOH methanol is used to measure chlorophyll b and β-Caroten (absorption wavelength of 445 nm) was measured and calculated using the following formula.

$$C = \frac{V \times A \times F \times 10}{2500}$$

C=Pigment levels in milligrams per g wet tissue.

V= Volume of extract

A= Absorption

F=1 can be considered

Hill reaction rate method for moldavian dragonhead Trebst (2000), using phosphate buffer was determined at a wavelength of 600 nm. Data records were analyzed statistically using SAS analysis of variance technique and LSD's Multiple Range Test at 5% probability level to compare differences among treatment means.

3. RESULTS AND DISCUSSION

3.1. Contents of chlorophyll a, b and a+b

The results showed that, of simple, double and triple drought stress, SA and JA on chlorophyll α content was significant at the one percent level (Tabele1). Such that maximum amount chlorophyll α in no stress levels with SA and JA (5.06 μg/mL) were observed (Fig.I). Chlorophyll α content decreased with increasing drought stress, and while the simple effect of SA (3.89 μg/mL) and JA (3.71 μg/mL) has been shown to increase the amount of chlorophyll α (Table 2). The amount of chlorophyll α with chlorophyll b, α+b and Hill reaction rate correlation is significant at the one percent level, But with β-carotene negative correlation between the surface of one percent (Table 4). The effects tested factors of simple (drought, SA and JA) on chlorophyll b was significant at of one percent level (Table1). So that the maximum amount of chlorophyll b in without stress conditions (1.47 μg/mL), salicylic acid 0.5 mM (1.67μg/mL) and jasmonic acid 50 μM (1.42μg/mL) was observed (Table 2). Jasmonic acid on interaction drought stress and salicylat on chlorophyll b is not significant. Also the effects of Triple factors tested (drought, salicylic acid and jasmonic acid) on a chlorophyll b wasnot significant (Table 1). The amount of chlorophyllb with chlorophyllα, α+b and Hill reaction rate correlation is

significant at the one percent level, and with Xanthophyll and β-carotene negative correlation between the surface of one percent (Table 4).
According to the analysis variance of table, significant at 1% level between the different levels of chlorophyll α+b in the amount of stress, salicylic acid and jasmonic acid there (Table1). So that comparisons between different levels of stress, jasmonic acid and salicylic acid showed the greatest amount of stress condition (3.72 μg/mL), jasmonic acid (5.13 μg/mL) and salicylic acid (5.56 μg/mL) chlorophyll α+β level was (Table 2). Interaction of Triple experimental factors affect the amount of chlorophyll wasnot significant (Table 2). Chlorophyll α+b has a positive correlation with content of chlorophyll α , b and Hill reaction rate is at 1%, However, the correlation coefficient content of β-carotene is the 1% level (Table 4).
The results of the study indicated that plant pigments have been affected by drought stress. The amount of chlorophyll a, b, total chlorophyll and photosynthetic reduced along with decrease in water potential (Table 2), which previously has been reported in numerous species such as oleander (*Nerium aleander*) and peas (Asadi kavan *et al.,* 2010). This may be due to increased activity of the stress chlorophyllasea plants, chlorophyll a, chlorophyll b and photosynthetic were also more susceptible to damage (Asadi kavan *et al.,* 2010). On the other hand, reactive oxygen species that are produced during stress, reduces the photosynthetic pigments (Sairam *et al.,* 2008). In the present study, treatment with salicylic acid was effective stress increase in chlorophyll content (Table 2). Therefore, the decrease in chlorophyll content and photosynthetic in dry conditions because of increased oxygen radicals. Peroxidation of the pigments and the chemical analysis cause genes to be (Wise & Nuyler, 2007). In this respect, the findings Loggini *et al.,* (1999), based on the outcome of the limitation of photosynthesis due to drought, the plants are exposed to excess energy. Since the excessive regenerative response center is also increased ROS production in chloroplasts. Thus, the expression Mahbub Alam *et al.,* (2013) can be reduced by reducing the amount of chlorophyll and photosynthetic in the leaves absorbs energy gained. According to this subject the increase in chlorophyll content in moldavian dragonhead exposed to drought in response to external SA can be linked to the induction of antioxidant that protects the plant against damage.

3.2. Contents of β-carotene and Xanthophyll

There was a significant difference at 1% level between the levels of stress, SA and JA (Table 1). So the most severe stress conditions β-carotene, without salicylic acid and JA content, 1.83, 2.09 and 1.97 (μg/mL) was observed respectively (Table 2). Also, interaction of Triple factors on agronomic characters experiment showed significant differences at 1% level (Fig. II). double effects of drought on jasmonic acid β-carotene was significant at the 5% level (Table1). maximum amount β-carotene severe drought conditions without SAand JA the amount were 2.96 (μg/mL) (Table 6). Also, the correlation coefficient β-carotene with chlorophyll a and b, a+b and hill reaction rate is negative and significant at the 1% level (Table 4). Results obtained of the analysis of variance table 1 showed that, The simple and double effects stress, SA and JA on contents of Xanthophyll was significant at the 1% level, but triple in significant at the 5% level (Table 1). maximum amount Xanthophyll severe drought conditions without salicylic acid and jasmonic acid the amount were 3.67 (μg/mL) (Fig. III). Xanthophyll With chlorophyll α+b, Hill reaction rate has a negative correlation coefficient at 1% level is the a positive correlation coefficient (Table 4).
According to experiments, the loss of chlorophyll and the Hill reaction rate, carotenoids (β-Caroten and Xanthophyll) increased (Table 2). This is in agreement with the results of Lawlor & Cornice (2002), which is expressed as β-Caroten and Xanthophyll pigments help effective. Another important role in the protection of membranes thylakoid, and also serve to prevent the photooxidation of chlorophyll (Lawlor & Cornice 2002). Mild water shortage will increase β-Caroten and Xanthophyll. Carotenoids, using Xanthophyll cycle and epoxidase and de-epoxidase reactions, oxygen consumption decreases and protects against photooxidation chlorophyll (Jeyaramraja *et al.*, 2005). Foliar application SA has been able to increase the amount of carotenoids (β-Caroten and Xanthophyll) (Table 2). In this regard, Viola xanthine de-peroxidase enzyme (VDE), catalyzed by the violaxanthin with zeaxanthin (Xanthophyll cycle) for activities that require ascorbate and salicylate (Muller_moule *et al.,* 2002). The relationship between Xanthophyll cycle, salicylate and redox status are well known. Excess electrons and active oxygen species that cause membrane damage thylakoid are protected by β-Caroten and Xanthophyll cycle, and the water deficit helps the survival of photosynthetic systems (Jagtap & Bhargara, 1995).

3.3. Hill reaction rate

The results showed that, of simple and double drought stress, SA and JA on Hill reaction rate was significant at the one percent level (Table1). Such that maximum amount Hill reaction rate in without stress (1.16 %OD/min), SA (0.93 %OD/min) and JA (0.90 %OD/min) were observed (Table 2). Hill reaction rate decreased with increasing drought stress (Table 2). Also, Interaction of Triple experimental factors affect the amount of Hill reaction rate was not significant (Table 1).The amount of Hill reaction rate with chlorophyllα, b and α+b correlation is significant at the one percent level, But with β-caroten negative correlation between the surface of one percent (Table 4).
Growth and photosynthesis under different environmental conditions such as drought takes effect. Factors limiting photosynthesis under drought stress has

been placed in two groups. A- Factors limiting stomatal: that stomatal closure in drought, reduced carbon dioxide (CO_2) concentration in leaves. Therefore less transferred to the chloroplast and photosynthesis (Hill reaction rate) decreases. B- Biochemical factors in photosynthesis: Such as chlorophyll content and enzyme activity rubisco, photosynthetic electron transport, hotophosphorylation, and the metabolites (Lawlor, 2002). According to the results of experiments, the effect of drought stress, photosynthesis rate (the rate of the Hill reaction) *Deracocephalum moldavica* L. is reduced. That seems to be due to reduced chlorophyll content. SA has a role in increasing the rate of photosynthesis and chlorophyll content (Belkhadi *et al.,* 2010). On the other hand, seems to spray salicylate with increasing power antioxidant moldavian dragonhead including β-Caroten and Xanthophyll reduces the amount of lipid peroxidation, H_2O_2, the more protection of cell membranes and photosynthesis, pigment of photosynthesis and the chlorophyll catabolism is prevented (Costa *et al.,* 2005). In this study, using jasmonic acid (JA) increased chlorophyll content in *Deracocephalum moldavica* L.. Different conclusions about the role of JA on photosynthetic pigments are listed. It is reported that, JA had no effect on chlorophyll content and photosynthesis in *Populus* is not (Babst *et al.,* 2005). Whereas, Jung (2004) has shown that *Arabidopsis thaliana*, 7 days after treatment with 100 μM JA concentration, chlorophyll content a, b decreased, and the amount of PSII electron transport is also affected. Weidhase *et al.,* (2007) also decreased chlorophyll content, and reduce the amount Rubisco in oat leaves JA treatment is indicated. Although several reports regarding the role of JA in the destruction of photosynthetic pigments is presented, but Ueda and Saniewski (2006) reported that the tulips, in the presence of light and chlorophyll a, b and a+b using JA formation is stimulated, resulting in the Hill reaction rate increased. The researchers stated that, JA on the expression of a series of key genes involved in the biosynthesis of chlorophyll by aminolevulinic acid. However, it has been observed at low concentrations of JA (Ueda & Saniewski, 2006). Czerpak *et al.,* (2006) also reported the alga *Chlorella valgaris*, causes the accumulation of chlorophyll has been JA. In another study, it has been reported that JA at concentrations of 0.1 μM repaired the photosynthetic pigment, including chlorophyll a and b, total chlorophyll and carotenoids (β-Caroten and Xanthophyll) in aquatic plant *Wolffia arrhiza* (*Lemnaceae*) (Piotrowska *et al.,* 2009). It is also shown that spray JA in terms of paraquat oxidative stress, and increased protection of photosynthetic chlorophyll in the plant oat has been (Popova *et al.,* 2003).

CONCLUSION

According to the results of this study appear to increase drought caused by oxidative stress and reduced chlorophyll a, b and a+b content, and is followed by a decrease in photosynthesis (Hill reaction rate). Also, increases the amount of auxiliary pigments (β-Caroten and Xanthophyll) as a non-enzymatic defense system, to overcome this stress is not efficient. It appears that the use of JA and SA increased antioxidant and non-antioxidant defense is power, so the effects of abiotic stresses in plants reduce Moldavian dragonhead .

Table 1.

Analysis variance of drought stress, salicylic acid and jasmonic acid on experimented traits

S. O.V	df	chlorophyllα content	Chlorophyllb content	Chlorophyll α+b content	β-carotene content	Xanthophyll content	Hill reaction rate
Drought	2	5.003**	0.78**	9.27**	9.71**	0.97**	1.80**
salicylic acid	1	8.43**	1.87**	18.25**	2.43**	11.88**	0.39**
jasmonic acid	1	17.52**	9.47**	52.75**	1.33**	6.72**	0.69**
Drought× salicylic acid	2	0.19ns	0.04ns	0.36*	1.11**	0.07*	0.07**
Drought× jasmonic acid	2	0.12ns	0.08ns	0.19ns	0.40**	0.04ns	0.04**
salicylic acid × jasmonic acid	1	0.40*	2.001**	0.62*	0.50**	2.78**	0.07**
Drought× salicylic acid × jasmonic acid	2	0.16*	0.03ns	0.06ns	0.12*	0.08**	0.01ns
Error	33	0.08	0.04	0.1	0.03	0.01	0.006
Coefficient Variation (%)		8.55	16.34	6.85	9.44	7.55	9.59

ns , * and **: Nonsignificant and significant at %5 and %1 level of probability respectively

Table 2.

Mean comparison simple effect of drought stress, salicylic acid and jasmonic acid on experimented traits

	chlorophyllα content (μg/mL)	Chlorophyll b content (μg/mL)	Chlorophyll α+b content (μg/mL)	β-carotene content (μg/mL)	Xanthophyll content (μg/mL)	Hill reaction rate (%OD/min)
Drought						
FC	3.77a	1.47a	5.24a	1.09c	1.35c	1.16a
2/3FC	3.41a	1.15b	4.56b	1.52b	1.61b	0.79b
1/3FC	2.68b	1.05b	3.72c	2.60a	1.83a	0.49c
jasmonic acid						
0mmol	2.87b	1.03b	3.89b	1.51b	1.09b	0.72b
50μmol	3.71a	1.42a	5.12a	1.96a	2.09a	0.90a
salicylic acid						
0mmol	2.68b	0.78b	3.46b	1.57b	1.22b	0.69b
0.5mmol	3.89a	1.67a	5.56a	1.90a	1.97a	0.93a

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test in 5% level of probability

Table 3.

Mean comparison of double interaction effects drought stress and jasmonic acid on experimented traits

	Chlorophyll α content (μg/mL)	Chlorophyll b content (μg/mL)	Chlorophyll α+b content (μg/mL)	β-carotene content (μg/mL)	Xanthophyll content (μg/mL)	Hill reaction rate (%OD/min)
FC+JA(0mM)	3.29cd	1.28b	4.57b	1.04e	0.77e	1.11b
FC+JA(50μM)	4.26a	1.66a	5.92a	1.14d	1.90b	1.21a
2/3FC+JA(0mM)	3.12d	1.00c	4.12bc	1.42c	1.12d	0.74cd
2/3FC+JA(50μM)	3.71b	1.30b	5.01ab	1.62b	2.10ab	0.84bc
1/3FC+JA(0mM)	2.20cd	0.80d	2.99c	2.01ab	1.39c	0.32e
1/3FC+JA(50μM)	3.15d	1.30b	4.45b	3.13a	2.27a	0.66d

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test in 5% level of probability

Table 4.

Mean comparison interaction effects of drought stress and salicylic acid on experimented traits

	chlorophyllα content (μg/mL)	chlorophyllb content (μg/mL)	Chlorophyll α+b content(μg/mL)	β-carotene content (μg/mL)	Xanthophyll content (μg/mL)	Hill reaction rate (%OD/min)
FC+SA(0mM)	3.13bc	0.95cd	4.08c	1.04d	1.02d	1.08ab
FC+ SA(0.5mM)	4.42a	1.99a	6.41a	1.14c	1.66b	1.23a
2/3FC+ SA (0mM)	2.75c	0.78cd	3.52d	1.42ab	1.21cd	0.62c
2/3FC+ SA(0.5mM)	4.08ab	1.53b	5.60ab	1.62ab	2.01ab	0.96b
1/3FC+ SA (0mM)	2.17d	0.61d	2.78e	2.25b	1.43bc	0.35d
1/3FC+ SA (0.5mM)	3.18b	1.49c	4.67b	2.95a	2.23a	0.60c

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test in 5% level of probability

Table 5.

Mean comparison of double interaction effects salicylic acid and jasmonic acid on experimented traits

	chlorophyllα content (μg/mL)	chlorophyllb content (μg/mL)	Chlorophyll α+b content(μg/mL)	β-carotene content (μg/mL)	Xanthophyll content (μg/mL)	Hill reaction rate (%OD/min)
$JA_{(0\mu M)}+SA_{(0mM)}$	2.35b	0.38c	2.73d	1.44c	0.96d	0.64c
$JA_{(0\mu M)}+SA_{(0.5mM)}$	3.38b	1.67a	5.05b	1.57bc	1.23c	0.80b
$JA_{(50\mu M)}+SA_{(0mM)}$	3.01c	1.18b	4.19c	1.69b	1.47b	0.74b
$JA_{(50\mu M)}+SA_{(0.5mM)}$	4.40a	1.66a	6.06a	2.23a	2.70a	1.06a

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test in 5% level of probability

Table 6.

Trait Correlation of *D.moldavica*

	Chlorophyll α content (μg/mL)	**Chlorophyll b content (μg/mL)**	**Chlorophyll α+b content(μg/mL)**	**β-carotene content (μg/mL)**	**Xanthophyll content (μg/mL)**	**Hill reaction rate (%OD/min)**
Chlorophyll α content (μg/mL)	1					
Chlorophyll b content (μg/mL)	0.72**	1				
Chlorophyll α+b content(μg/mL)	0.96**	0.89**	1			
β-carotene content (μg/mL)	-0.13ns	-0.02ns	-0.08ns	1		
Xanthophyll content (μg/mL)	-0.53**	-0.44**	-0.53**	0.59**	1	
Hill reaction rate (%OD/min)	0.78**	0.58**	0.75**	-0.44**	-0.18ns	1

Ns Nonsignificant, * Significant at 5% probability and ** Significant at 1% probability

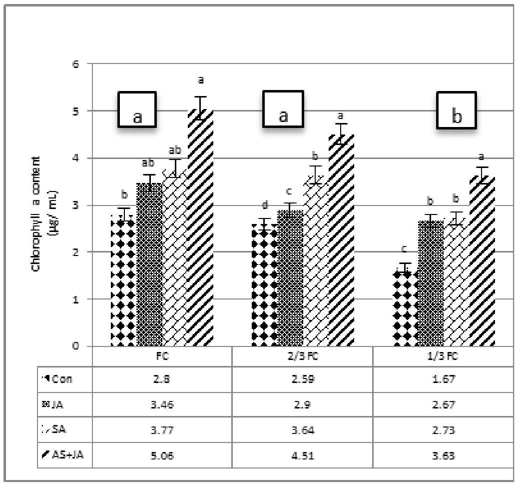

Fig. I. Interaction of effects of drought, jasmonic acid and salicylic acid on Chlorophyllα content

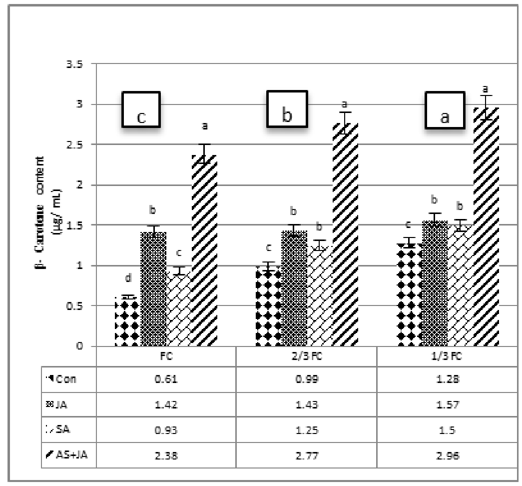

Fig. II. Interaction of effects of drought, jasmonic acid and salicylic acid on β-Carotene content

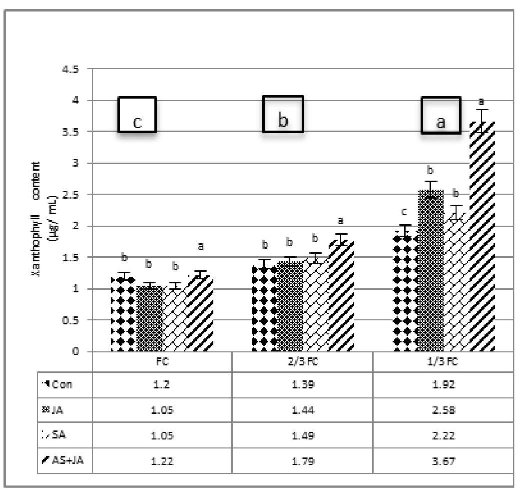

Fig. III. Interaction of effects of drought, jasmonic acid and salicylic acid on Xanthophyll content

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