Journal of Plant Process and Function, Vol. 7, No. 25, Year 2018

The effect of spraying of methyl jasmonate and Epi-brassinolide on photosynthesis, chlorophyll fluorescence and leaf stomatal traits in black mustard (*Brassica nigra* L.) under salinity

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(Received: 23/10/2017-Accepted: 18/04/2018)

Abstract

Methyl jasmonate and Epi-brassinolide as plant growth regulators have significant biological effects on plant growth, including increasing of tolerance to salt stress in plants. In this research, the effects of salinity stress and its interaction with methyl jasmonate and Epi-brassinolide on chlorophyll concentration, rate of photosynthesis, transpiration, stomatal conductance and resistance and chlorophyll fluorescence in medical plant of black mustard (*Brassica nigra* L.) in hydroponic conditions, were evaluated based on a factorial design in completely randomized design with four replications in 2015, at the Damghan branch of Islamic Azad University was studied. Plants were treated with various concentrations of sodium chloride (0, 40, 80 mM), methyl jasmonate (0 and 75 mM) and Epi-brassinolide (0, 5.1, 3 mM) four weeks after germination. The plants treated with salinity, with increasing concentration of sodium chloride, their chlorophyll concentrations, Hill reaction rate, transpiration and stomatal conductance decreased but the stomatal resistance and maximum fluorescence (Fm) increased. At the same amounts of NaCl, with increasing concentrations of methyl jasmonate and Epi-brassinolide all tested traits were improved, such that by spraying 75 mM of methyl jasmonate, stomatal conductance (90.23 $m^2 s\ mol^{-1}$) reached its highest level. With increasing levels of Epi-brassinolide in the same condition, the salinity of maximum fluorescence (Fm) reached to (559.21). The results of this experiment showed that the use of methyl jasmonate and 24-epibrassinosteroids can reduce stomatal resistance and increase stomatal conductance, chlorophyll concentration and improve the rate of photosynthesis and thus reduce the effects of salt stress in black mustard (*Brassica nigra* L.).

Keywords: Black mustard (*Brassica nigra* L.), Chlorophyll fluorescence and Stomatal conductance

Introduction

Black mustard (*Brassica nigra* L.) is, of wallflower race, herbaceous, annual, 0.5 to 1.5 meters in height, and its shoots are bluish green, and covered with fluff in the lower parts of plant. Its leaf blade has cuts. With yellow flowers and fruits to 1.5 to 2.5 cm long and has lead to thin and short beak. Black mustard plant has many uses in traditional medicine and the therapeutic value of black mustard seeds in external use is much more than internal consumption. In its external use, its Round grain is used to relieve neuralgia, rheumatism and relieve choking (Tawaha and Turk, 2003)

Salinity is the ability to limit the construction of plants in the soil medium in large areas around the world. Efforts to expand the plant resistance to stress is very important. For that purpose it will increase crop production (Arshad Niji *et al.*, 2016).

Increase in stress resistance in plants treated is seen with Brassinolides (Faiduddin *et al*., 2014). Brassinolides are a group of plant hormones with significant biological effects on plant growth, including increasing the resistance of plants to environmental stresses. Brassinolides can act by reducing the amount of reactive oxygen produced by the environmental stress with the help of enzymatic and non-enzymatic mechanisms and protects the integrity of biological membranes and increases the yield of vegetable crops (Marco *et al*., 2014). Jasmonates (the jasmonic acid and its methyl ester, methyl jasmonate) are a new class of plant growth regulators that participate in many physiological processes of the plant and play a defensive role (Srivastava, 2002). Application of methyl jasmonate and NaCl simultaneously to deal with salinity stress in tomatoes was helpful. Thus, the effects of toxic ions that have been created by salinity, can cause damage to the cell membrane and causes the release of lipids for the synthesis of jasmonate (Seif *et al*., 2014). In addition, Parra-Lobato and colleagues (2009) concluded that the use of external methyl jasmonate may indicate its function in the oxidative stress processes regulating internal antioxidant enzymes.

Therefore, due to increasing expansion of saline land, the purpose of this study, in addition to evaluation of photosynthesis in black mustard plants, is

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investigation of protection of methyl jasmonate and Brassinolides and also its interaction against oxidative stress caused by salinity of sodium chloride.

Material and Methods: In order to evaluate the effect of spraying of methyl jasmonate and Epi-brassinolide on photosynthesis, chlorophyll fluorescence and leaf stomatal traits in black mustard (*B. nigra*) under salinity a factorial experiment was carried out in the Damghan branch of Islamic Azad University in 2015 in a completely randomized in 3 replications. Culture medium was prepared in a hydroponic manner. A large number of seeds per pot were planted that after growth, 7 plant remained for treatment during two stages of thinning. The treatments included three levels of salinity (0, 40 and 80 mM), methyl jasmonate treatments (0 and 75 Mμ) and Epi-brassinolide (0, 1.5 and 3 Mμ). 3 weeks after the start of salinity stress traits were measured.

Chlorophyll fluorescence with GFP device: To measure the fluorescence of leaves the chlorophyll fluorescence measurement device (GFP) (GFL-1, OPTI-SCIENCES, CCM-200, USA) model Plant stress meter was used. At the appropriate time of evaluating the parameters after the last stress applied and hormone spray considered, the last fully mature leaf in about the same position in each sample was covered by aluminum foil for 30 minutes and was adapted to the darkness. Then the sensor of chlorophyll fluorescence device (GFP) was attached and by turning the device on, the light at a wavelength of 695 nm radiated to the leaf through an optical fiber. Fluorescence parameters, including minimum fluorescence (F0) and maximum fluorescence (Fm) were measured. The light level was selected as the photon flux density (PFD) of 400 mmol photons per meter per second and the light radiation time was 5 seconds for all treatments which was repeated 10 times on each treatment. Maximum quantum yield of PS (II) (Fv/Fm) and variable fluorescence (Fv) were calculated from the following relationship.

$(F_v/F_m) = (F_m-F_0)/F_m$ $F_v= F_m-F_0$

Hill reaction measurement in chloroplasts: One gram of tissue was removed and was homogenized with 3ml of phosphate buffer at pH=7 and at around 5000 were centrifuged for 15 minutes, then sediments were separated from it and 2ml of chloride Tris buffer was added to it. After homogenization, 0.5ml of the buffer was mixed with 0.3ml of dichlorophenol indole phenol and 0.3ml of distilled water, then changes in absorbance at intervals of 1 minute at a wavelength of 600 nm were measured with the spectrophotometer model (Jenway Genova) and the mean absorbance changes are expressed as Hill reaction rate (Trebst, 1872).

Stomatal traits (stomatal conductance, transpiration, stomatal resistance) were studied with the help of Lcpro device. For this purpose, five fully mature leaves with the same age were sampled from each pot and stomatal conductance, transpiration, stomatal resistance were calculated by Lcpro device.

Measurement of chlorophyll concentrations: To measure chlorophyll concentration chlorophyll meter SPAD-502 (OPTI-SCIENCES, CCM-200, USA) was used.

Results

Chlorophyll meter value: The results showed that in terms of chlorophyll meter value there was a significant difference between different levels of salinity at the level of 1% (Table 1). In this case, the average concentration of chlorophyll in a state of extreme salinity stress (80mM) reached to 39.61, in the medium salinity (40mM) it had the value of 47.69 and, in non-stress conditions 39.59 (table 2). The effect of Jasmonate levels on chlorophyll concentration was significant at 1% level (Table 1). So that the application of 75μM of methyl jasmonate with 60.27 had the highest concentration of chlorophyll and the non-application of methyl jasmonate had the lowest with 37.52 (Table 2). Effect of different levels of Brassinolides on chlorophyll meter value was significant at 1% level (Table 1). In this case, the average concentration of chlorophyll in the case of using Brassinolide with the concentration of 3μM had the highest number of chlorophyll-meters (58.86), at a concentration of 5.1μM reached 47.13 and in case of non-application of Brassinolide 40.69 (table 2). Interaction of salinity and methyl jasmonate and salinity stress and Brassinolide on chlorophyll concentration was not significant (Table 1). Interaction of Brassinolide and methyl jasmonate on chlorophyll meter value was significant at 1% level (Table 1). Comparison of interaction of these two factors on the discussed trait showed that applying the conditions of using Brassinolide and application of 75μM methyl jasmonate had the highest concentration of chlorophyll with the value of 76.18 (Table 3). Triple interaction effect (salinity × methyl jasmonate × Epi-brassinolide) on the chlorophyll content of a + b was not significant (Table 1).

Hill reaction rate: The results showed that, in terms of Hill reaction rate, between different levels of salinity stress significant difference at 1% level was observed (Table 1). In this case, the average rate of Hill reaction in a state of extreme stress (80mM) reached 0.55 (%OD min^{-1}), in medium salinity stress had (40mM) 0.064 (%OD min^{-1}), and in no stress conditions it had the value of 0.69 (%OD min^{-1}), (table 2). The different levels of jasmonate on Hill reaction rate was significant at 1% level (Table 1). So that the application of 75μM methyl jasmonate with 0.75 (%OD min^{-1}) had the highest Hill reaction rate and the non-application of methyl jasmonate with 0.50 (%OD min^{-1}) had the lowest Hill reaction rate (Table 2). Effect of different levels of Brassinolide on the Hill reaction rate was significant at 1% level (Table 1). In these conditions average Hill reaction in the case of using Brassinolide with the concentration of 3μM had the highest rate of xanthophyll (0.83 (%OD min^{-1})), at the concentration of

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Table 1. Analysis variance of salinity stress, Epi-brassinolide and methyl Jasmonate on experimented traits

S.O.V	df	Stomatal conductance	Stomatal resistance	Transpiration	chlorophyll concentration	Hill reaction rate	F_0	F_m	F_v	F_v/F_m
Salt	2	3522**	1392577^{**}	2731^{**}	2374.15^{**}	0.13^{**}	32415^{ns}	23548^{**}	424.1^{ns}	0.02^{ns}
Ja	1	9734**	22432718^{**}	1819^{**}	9319.77^{**}	1.06^{**}	32511^{ns}	37779*	454.9^{ns}	0.04^{ns}
Br	2	5447**	20663589^{**}	3186^{**}	2035.15^{**}	1.30^{**}	65584^{ns}	4339^{ns}	245.1^{ns}	0.05^{ns}
Ja × Sa	2	38^{ns}	505044^{**}	207^{**}	76.21^{ns}	0.0002^{ns}	5544^{ns}	79544^{**}	542.2^{ns}	0.01^{ns}
Br × Sa	4	77^{ns}	3276750^{**}	607^{**}	12.67^{ns}	0.006^{ns}	41895^{ns}	13602^{**}	455.4^{ns}	0.1^{ns}
Br × Ja	2	2278**	6556697^{**}	43.25^{ns}	669.43^{**}	0.032^{**}	25485^{ns}	93479^{**}	555.4^{ns}	0.071^{ns}
Br× Ja×Sa	4	101^{ns}	1947267^{**}	249^{**}	35.17^{ns}	0.007^{ns}	5444^{ns}	59116^{**}	585.9^{ns}	0.07^{ns}
Error	54	66.89	20887	19.93	25.28	0.004	5554.75	4638.12	547.12	0.08
C.V		10.37	5.53	10.62	10.28	9.60	7.35	15.58	9.01	8.48

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

Table 2. Mean comparison simple effect of salinity stress, Epi-brassinolide and methyl Jasmonate on experimented traits

	Stomatal conductance	Stomatal resistance	Transpiration	chlorophyll meter value	Hill reaction rate	F_0	F_m	F_v	F_v/F_m
	$m^2s\ mol^{-1}$	$S\ m^{-1}$	$mmol\ m^2s^{-1}$	SPAD value	$\%OD\ min^{-1}$				
Salinity									
0 mmol	89.30^a	2356.79^c	29.87^a	59.39^a	0.69^a	96.09^a	350.87^c	254.58^a	0.73^a
40 mmol	81.81^b	2649.67^b	25.48^b	47.69^b	0.64^b	105.65^a	403.91^b	298.26^a	0.74^a
80 mmol	65.60^c	2834.50^a	9.59^c	39.61^c	0.55^c	127.65^a	442.69^a	315.04^a	0.71^a
methyl asmonate									
0 µmol	67.24^b	3171.83^a	16.62^b	37.52^b	0.50^b	135.69^a	455.40^a	319.71^a	0.70^a
75 µmol	90.23^a	2055.47^b	26.67^a	60.27^a	0.75^a	73.62^a	409.58^b	335.96^a	0.85^a
Epi-brassinolide									
0 µmol	61.85c	3672.63^a	8.74^c	40.69^c	0.38^c	99.09^a	434.68^a	335.59^a	0.82^a
1.5 µmol	84.67b	2225.29^b	25.31b	47.13^b	0.67^b	124.67^a	418.08^a	293.41^a	0.70^a
3 µmol	90.39a	1943.04^c	30.88^a	58.86^a	0.83^a	159.21^a	444.71^a	285.50^a	0.64a

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 Epi-brassinolide and methyl Jasmonate on experimented traits

Ja×Br	Stomatal conductance	Stomatal resistance	Transpiration	chlorophyll meter value	Hill reaction rate	F_0	F_m	F_v	F_v/F_m
	$m^2s\ mol^{-1}$	$S\ m^{-1}$	$mmol\ m^2s^{-1}$	SPAD value	$\%OD\ min^{-1}$				
Ja0mM×Br0µM	38.99^c	4834^c	2.34^d	33.46^d	0.22^d	74.68^{cd}	529^a	454^a	0.86^a
Ja0mM×Br1.5µM	77.76^b	2497^b	20.35^{bc}	37.56^d	0.58^c	96.18^{bc}	402^{ab}	306^b	0.76^b
Ja0mM×Br3µM	85.07^{ab}	2184^{bc}	27.17^{ab}	41.53^{cd}	0.70^b	104.00^b	433^{ab}	329^{ab}	0.76^b
Ja75mM×Br0µM	85.70^{ab}	2511^b	15.13^c	47.93^c	0.50^c	212.50^d	339^b	127^c	0.38^c
Ja75mM×Br1.5µM	91.18^a	1953^{bc}	30.30^{ab}	56.70^b	0.74^b	280.17^a	433^{ab}	153^c	0.35^c
Ja75mM×Br3µM	95.71^a	1701^a	34.60^a	76.18^a	0.97^a	314.42^a	455^{ab}	158^a	0.36^c

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

1.5µM and in case of non-application of Brassinolide, it reached the value of 0.67 and 0.38 ($\%OD\ min^{-1}$), respectively (table 2). Interaction of salinity and methyl jasmonate on Hill reaction rate was significant at 1% level (Table 1). Comparison of average interaction of these two factors on the discussed traits showed that application of salinity conditions and 75µM methyl jasmonate with 0.67 ($\%OD\ min^{-1}$) had the highest Hill reaction rate (Table 4). Dual interaction effect between salinity stress × Brassinolide was not statistically

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Table 4- Mean comparison of double interaction effects salinity stress and methyl Jasmonate on experimented traits

Sa×Ja	Stomatal conductance	Stomatal resistance	Transpiration	chlorophyll meter value	Hill reaction rate	F_0	F_m	F_v	F_v/F_m
	$m^2s\ mol^{-1}$	$S\ m^{-1}$	$mmol\ m^2s^{-1}$	SPAD value	$\%OD\ min^{-1}$				
Sa0mM×Ja0µM	76.75^{c}	2792.58^{a}	23.06^{ab}	45.97^{d}	0.67^{d}	95.66^{cd}	324.15^{a}	228.49^{b}	0.70^{c}
Sa0mM×Ja75µM	101.84^{b}	1921.00^{b}	36.68^{a}	72.81^{d}	0.81^{c}	128.42^{bc}	377.58^{ab}	249.16^{a}	1.94^{a}
Sa40mM×Ja0µM	69.66^{ab}	3368.08^{bc}	23.85^{ab}	37.13^{cd}	0.52^{b}	193.92^{b}	413.32^{ab}	219.40^{b}	1.13^{b}
Sa40mM×Ja75µM	93.96^{ab}	1931.25^{b}	27.12^{a}	58.26^{c}	0.76^{c}	212.42^{d}	394.50^{b}	182.08^{c}	0.86^{c}
Sa80mM×Ja0µM	55.41^{a}	3354.83^{bc}	20.96^{ab}	29.46^{b}	0.44^{b}	221.28^{a}	628.72^{ab}	407.44^{a}	1.84^{a}
Sa80mM×Ja75µM	75.79^{a}	2314.17^{c}	16.23^{c}	49.75^{a}	0.67^{a}	238.75^{a}	456.67^{ab}	227.97^{a}	0.95^{a}

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 salinity stress and Epi-brassinolide on experimented traits

Sa×Br	Stomatal conductance	Stomatal resistance	Transpiration	chlorophyll meter value	Hill reaction rate	F_0	F_m	F_v	F_v/F_m
	$m^2s\ mol^{-1}$	$S\ m^{-1}$	$mmol\ m^2s^{-1}$	SPAD value	$\%OD\ min^{-1}$				
Sa0mM×Br0µM	75.87^{c-e}	2662.38^{b}	7.14^{c}	50.36^{bc}	0.46^{d}	64.60^{e}	222.73^{c}	158.13^{c}	0.71^{b}
Sa0mM×Br1.5µM	93.25^{a-c}	2278.50^{bc}	37.27^{ab}	56.91^{ab}	0.70^{bc}	137.64^{cd}	407.87^{b}	270.23^{c}	0.66^{b}
Sa0mM×Br3µM	97.78^{a}	2129.50^{bc}	42.20^{a}	70.89^{a}	0.91^{a}	183.88^{ab}	422.00^{b}	238.12^{c}	0.56^{b}
Sa40mM×Br0µM	61.24^{ef}	4422.75^{a}	12.30^{c}	39.34^{cd}	0.39^{de}	172.88^{d}	371.98^{b}	199.90^{c}	2.15^{a}
Sa40mM×Br1.5µM	88.74^{a-d}	1897.50^{bc}	29.19^{b}	46.46^{b-d}	0.68^{c}	193.50^{ab}	408.62^{b}	214.50^{c}	0.52^{b}
Sa40mM×Br3µM	95.44^{ab}	1628.75^{c}	34.96^{b}	57.28^{ab}	0.85^{ab}	175.88^{ab}	431.13^{b}	255.12^{c}	0.59^{b}
Sa80mM×Br0µM	48.43^{f}	3932.75^{a}	6.77^{c}	32.39^{d}	0.28^{e}	$151.88b^{c}$	709.33^{a}	557.12^{b}	0.79^{b}
Sa80mM×Br1.5µM	71.42^{de}	2499.88^{bc}	9.51^{c}	38.01^{cd}	0.62^{c}	106.88^{ab}	737.75^{b}	630.87^{a}	0.86^{b}
Sa80mM×Br3µM	76.95^{b-e}	2070.88^{bc}	12.50^{c}	48.41^{bc}	0.75^{bc}	108.88^{a}	481.00^{b}	372.12^{ab}	0.77^{b}

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

significant (Table 1). The dual interaction effect of jasmonate × Epi-brassinolide on Hill reaction rate had a significant effect on the at 1% level (Table 1). The highest rate in methyl jasmonate with a concentration of 75µM and Brassinolide in the concentration of 3µM was 0.94 ($\%OD\ min^{-1}$) (Table 3). Triple interaction effect (salinity × methyl jasmonate × Epi-brassinolide) on the Hill reaction rate was not statistically significant (Table 1).

Stomatal conductance: The results showed that, in terms of stomatal conductance between the different levels of salinity stress significant difference at 1% level was observed (Table 1). In this situation average stomatal conductance in a state of extreme stress (80mM) reached to the value of 65.60 ($m^2s\ mol^{-1}$), in the medium salinity (40mM) was 81.81 ($m^2s\ mol^{-1}$) and in non-stress condition it was 89.30 ($m^2s\ mol^{-1}$) (table 2). The effect of different levels of jasmonate on stomatal conductance was significant at 1% level (Table 1). Application of 75µM methyl jasmonate with the value of 90.23 ($m^2s\ mol^{-1}$) was the maximum stomatal conductance and non-application of methyl jasmonate with the value of 67.24 ($m^2s\ mol^{-1}$) 24/67 was the lowest stomatal conductance (Table 2). Effect of different levels of Brassinolide on stomatal conductance was significant at 1% level (Table 1). In this condition the average stomatal conductance in the case of the use of Brassinolide with the concentration of 3µM had the highest xanthophyll concentration (90.39 ($m^2s\ mol^{-1}$)), and at a concentration of 1.5µM had the value of 84.67 ($m^2s\ mol^{-1}$) and in the case of not using Brassinolide it was 61.85 ($m^2s\ mol^{-1}$) (table 2). Interaction of salinity and methyl jasmonate and salinitystress × Brassinolide was not statistically significant (Table 1). The dual interaction of jasmonate × Epi-brassinolide had a significant effect on stomatal conductance at 1% level (Table 1). The highwst speed in the case of methyl jasmonate with a concentration of 75µM and Brassinolide at the concentration of 3µM had the value of 95.71 ($m^2s\ mol^{-1}$) (Table 3). Triple interaction effect (salinity × methyl jasmonate × Epi-brassinolide) on stomatal conductance was not statistically significant (Table 1).

Stomatal resistance: The results showed that, in terms of stomatal resistance there was a significant difference at 1% level between different salinity levels (Table 1). In this condition, the average stomatal conductance in the case of extreme stress (80mM) was 2834.50 ($s\ m^{-1}$), in the case of medium salinity (40mM) it was 2649.67 ($s\ m^{-1}$) and in non-stress condition it was 2356.79 ($s\ m^{-1}$) (table 2). The effect of different levels of jasmonate on stomatal resistance was significant at

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1% level (Table 1). The use of 75µM methyl jasmonate had the highest stomatal resistance of 2055.47 (s m^{-1}) and non-application of methyl jasmonate has the lowest stomatal resistance of 3171.83 (s m^{-1}) (Table 2). The effect of different levels of Brassinolide on stomatal resistance was significant at 1% level (Table 1). In this condition the average stomatal resistance in the case of using Brassinolide with the concentration of 3µM had the highest xanthophyll concentration (1943.04 (s m^{-1})), and at a concentration of 1.5µM had the value of 2225.29 (s m^{-1}) and in the case of not using Brassinolide it was 3672.63 (s m^{-1}) (table 2). Interaction effect of salinity and methyl jasmonate was significant at 1% level (Table 1). The highest stomatal resistance was in the condition of non-stress and the non-application of methyl jasmonate with the value of 2792.58 (s m^{-1}) (Table 4). Effect of Salinity stress × Brassinolide on stomatal resistance was significant at 1% level (Table 5). At 40mM salinity condition and non-application of Brassinolide the stomatal resistance was 4422.75 (s m^{-1}) (Table 5). Dual interaction effect of jasmonate × Epi-brassinolide on stomatal resistance was significant at 1% level (Table 1). The greatest resistance in the condition of methyl jasmonate with concentration of 75µM and Brassinolide with the concentration of 3µM was 1701.92 (s m^{-1}) (Table 3). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on stomatal resistance was also significant at 1% level (Table 1). However, the highest stomatal resistance in 80mM salinity conditions and non-use of methyl jasmonate and Epi-brassinolide reached the value of 5304.25 (s m^{-1}) (Figure 1).

Transpiration: The results showed that, in terms of transpiration between different levels of salinity, significant difference was observed at 1% level (Table 1). In this condition, the average transpiration in the case of extreme stress (80mM) was 9.59 (mmol m^2s^{-1}), in the case of medium salinity (40mM) it was 25.48 (s m^{-1}) and in non-stress condition it was 29.87 (mmol m^2s^{-1}) (table 2). The effect of different levels of jasmonate on transpiration was significant at 1% level (Table 1). The use of 75µM methyl jasmonate had the highest transpiration of 26.67 (mmol m^2s^{-1}) and non-application of methyl jasmonate had the lowest transpiration of 16.62 (mmol m^2s^{-1}) (Table 2). The effect of different levels of Brassinolide on transpiration was significant at 1% level (Table 1). In this condition the average transpiration rate in the case of using Brassinolide with the concentration of 3µM had the highest xanthophyll concentration (30.88 (mmol m^2s^{-1})), and at a concentration of 1.5µM had the value of 25.31 (mmol m^2s^{-1}) and in the case of not using Brassinolide, it was 8.74 (mmol m^2s^{-1}) (table 2). Interaction effect of salinity and methyl jasmonate was significant at 1% level (Table 1). The highest transpiration rate was in the condition of 80mM salinity stress and application of 75mM methyl jasmonate with the value of 16.23 (mmol m^2s^{-1}) (Table 5). Effect of Salinity stress × Brassinolide on stomatal transpiration was significant at 1% level (Table 5). In the condition of non-salinity and application of 3mM Brassinolide the transpiration rate was 42.20 (mmol m^2s^{-1}) (Table 5). Dual interaction effect of jasmonate × Epi-brassinolide on transpiration was significant at 1% level (Table 1). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on stomatal transpiration was also significant at 1% level (Table 1). However, the highest transpiration rate was observed in the condition non-salinity and application of methyl jasmonate with concentration of 75µM and Brassinolide with the concentration of 3µM with the value of 53.60 (mmol m^2s^{-1}) (Table 3).

The initial fluorescence (F0): The results showed that, in terms of fluorescence (F0) there was no significant difference between the different levels of salinity, brassinolide and jasmonate (Table 1). Dual interaction effect of salinity × methyl jasmonate, salinity × brassinolide and jasmonate × Epi-brassinolide was not significant (Table 1). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on terms of fluorescence (F0) was not significant (Table 1).

The maximum fluorescence (Fm): The results showed that, in terms of maximum fluorescence (Fm) there was a significant difference at 1% level between the different levels of brassinolide and jasmonate but the difference at different levels of Brassinolide was not significant (Table 1). In this condition, the maximum fluorescence rate (Fm) in a state of extreme stress (80mM) increased to 552.52 (Table 2). The simple effect of methyl jasmonate on maximum fluorescence (Fm) was 535.65 and for brassinolide with the concentration of 3µM it was 559.21 (Table 2). Dual interaction effect of salinity × methyl jasmonate, salinity × brassinolide and jasmonate × Epi-brassinolide on maximum fluorescence (Fm) was significant at 1% level (Table 1). At conditions of 80mM salinity stress with 3µM brassinolide the maximum fluorescence (Fm) was 580.88 (Table 5). Dual interaction of 75µM methyl jasmonate and brassinolide on the maximum fluorescence (Fm) reached the value of 614042 (Table 3). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on the maximum fluorescence (Fm) was also significant at 1% level (Table 1).

Quantum yield of photosystem II (Fv): The results showed that, in terms of quantum yield of photosystem II (Fv), there was no significant difference between the different levels of salinity, brassinolide and jasmonate (Table 1). Dual interaction effect of salinity × methyl jasmonate, salinity × brassinolide and jasmonate × Epi-brassinolide was not significant (Table 1). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on quantum yield of photosystem II (Fv) was not significant (Table 1).

Maximum quantum yield of photosystem II in the conditions adapted with darkness (Fv/Fm): The results showed that, in terms of maximum quantum yield of photosystem II in the conditions adapted with

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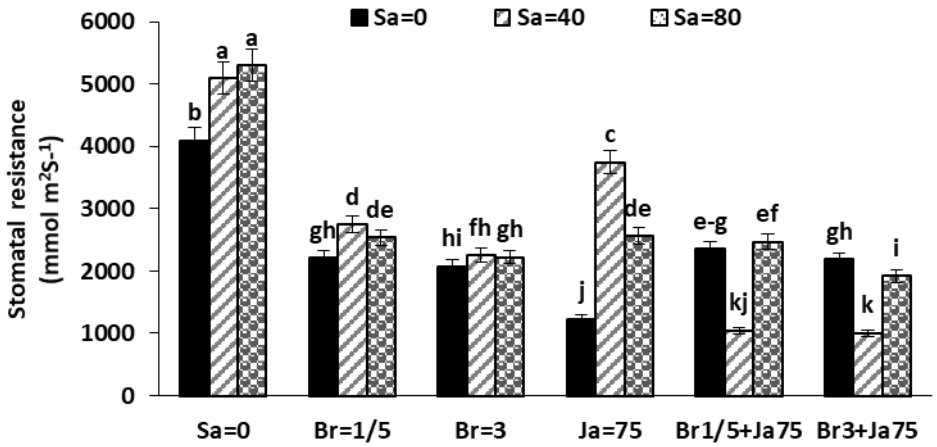

Fig 1. Interaction of salinity stress, epi-brassinolide and methyl Jasmonate treatment on the Stomatal resistance, Con (Control), Sa (salt), Br (Epi-brassinolide) and Ja (methyl Jasmonate). Results for Mean Mean ± SE by Duncan test (P≤0.05) is shown. Joint letters no significant difference in the level of 5%.

darkness (Fv/Fm) there was no significant difference between the different levels of salinity, brassinolide and jasmonate (Table 1). Dual interaction effect of salinity × methyl jasmonate, salinity × brassinolide and jasmonate × Epi-brassinolide was not significant (Table 1). Triple interaction effect of (salinity × methyl jasmonate × Epi-brassinolide) on maximum quantum yield of photosystem II in the conditions adapted with darkness (Fv/Fm) was not significant (Table 1).

Discussion

Sodium chloride reduces the concentration of chlorophyll which is essential for the main stages of photosynthesis and growth of cells and its rate. The lowest rate of photosynthesis and chlorophyll meter value in plants under NaCl = 80mM have been observed (Table 2). Although stomatal closure and increased stomatal resistance in stress conditions (Table 2) are conducted in order to reduce water loss and transpiration but it could reduce photosynthesis to less than the compensation by preventing the entry of CO2 (Ashraf and Harris, 2004). Turan *et al*. (2009) and Cha-um (2009) in the investigation of the corn plant under salinity and Capsicum (Bettaieb *et al*., 2008), stated that the entire content and concentration of chlorophyll treated, the salinity and stomatal resistance decrease and, photosynthesis increases. It has been shown that salinity stress leads to destruction, and changes in the number and size of chloroplasts. Reduced intensity of photosynthesis is caused by salinity stress that is due to several factors such as dehydration of cell membrane and thereby reducing the permeability of CO2, salt toxicity, reduced amount of CO2 due to stomatal closure, accelerated aging process as a result of salt, the change in activity of enzymes due to structural changes in the cytoplasm and negative feedback due to lower activity of the source. Salinity also prevents the photosynthetic electron transport, stomatal conductance reduction and increased production of active oxygen species that causes oxidative damage to photosystems (Munns, 2002). Reduced quantum yield in corn seedlings under salinity stress causes a decrease in net photosynthesis, which leads to a significant reduction in growth (Turan *et al*., 2008). Brassinolides can reduce active oxygen produced by the environmental stress with the help of increased enzymatic and non-enzymatic mechanisms causing the protection of the integrity of biological membranes and increase in the photosynthetic pigments and, thereby photosynthesis (Munns, 2002). Therefore, in accordance with Table 2 chlorophyll concentration and photosynthesis rate increase. In maize (Cha-um, 2009), *vica faba* (Bettaieb *et al*., 2008), canola (Kaouther et al., 2012) and *vitis viniferal* (Seif *et al*., 2014) at the time of oxidative stress in the cell they can be adjusted in the presence of Brassinolides. Brassinolide acts opposite abscisic acid (ABA). Brassinolide is able to inhibit the production of ABA. Abscisic acid is known as a stress hormone that reacts to environmental stresses associated with changes in water activity through the metabolic and developmental mechanisms. Plants react to the environmental stresses such as drought and salinity by varying the concentration of ABA. After its concentration exceeded the threshold level, it causes complete closure of stomata and changes in how genes are expressed. Therefore, the stomatal resistance increases and stomatal conductance and transpiration rate decreases. ABA has been increased due to salinity and as a signal in response to stress causes stomatal closure, which is as a defense mechanism to protect the water. ABA's negative effects can be removed by using Epi-brassinolide and stomatal resistance is reduced (Zhang *et al*., 2006). Brassinolide treatment under salt stress, increased stomatal conductance compared to the control and will help to enhance photosynthesis (Zhang *et al*., 2006). More than 99 percent of water absorbed by the roots of plants through transpiration, especially through leaf stomata is lost. Treatment of Robinia Pseudo avvacia with 0.2 mg of Brassinolide (Julie *et al*., 2011), leaves of wheat at two levels of Brassinolide

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(Fariduddin *et al*., 2014) and in corn (Rezaei, 2015), stomatal conductance and transpiration rate under salinity stress were improved using Brassinolide. The effect of this growth regulator on stomatal conductance is heavily dependent on species, type and severity of stress, as well as its concentration and application method (Rezaei, 2015). 75 micro-molar concentration of methyl jasmonate increases the amount of chlorophyll in B.nigra (Table 2). In the case of the effect of methyl jasmonate on the value of photosynthetic pigments different results have been listed. For example, it had no effect on the chlorophyll and photosynthesis of pine (Sadeghi Pour and Bonakdar Hashemi, 2015). While Jung in 2004 showed that in the Arabdoisis plant, 7 days after treatment with methyl jasmonate at a concentration of 100µM, chlorophyll a and b contents decreases and the electron transport of photosystem II is also affected. The presence of light and methyl jasmonate in tulips stimulates the formation of chlorophyll a and b (Qayyum *et al*., 2007). Weidhase and colleagues (1987) have also shown decreased chlorophyll content, photosynthetic dye degradation and reduced amount of RuBisCO in barley leaves treated with methyl jasmonate. Methyl jasmonate involved in the expression of a series of key enzymes in the biosynthesis of chlorophyll through the formation of lowelinik amino acid. However, this has been observed at low concentrations of methyl jasmonate (Qayyum *et al*., 2007). In the algae Chlorella vulgaris, jasmonate has caused the aggregation of chlorophyll (Anju metal, 2011). During the survey on black mustard plant, it has been shown that methyl jasmonate (75µM) has increased concentrations of chlorophyll and photosynthesis rate (Table 2). In another study it has been reported that jasmonate at a concentration of 0.1µM, repaired the photosynthetic pigments such as chlorophyll a and carotenoids in a blue Duckweed (Babs *et al.*, 2005) and barley under the herbicide Paraquat (Jung, 2004). Methyl jasmonate at concentrations up to 100 micromolar increases the ABA and ethylene in plants. Therefore, it can indirectly cause stomatal closure resulting in increased stomatal resistance and reduced transpiration rate. On the other hand, lead to activation of mitogen-activated protein kinase (MAPK) that impact stomata and an increase in the ABA in response to biotic and abiotic stresses. This MAPK is activated by upstream mitogen-activated protein kinase (MAPKK). Methyl jasmonate leads to activation of the MPK4 by inducting stress response genes. MPK4 causes to activate the MAPKK. MPK4 is expressed in the epidermis and stomatal sensitivity to changes in the environment. So spraying methyl jasmonate causes activation of MPK4 and thereby activation of protein kinase cascade pathway in the stomatal guard cells can lead to activation of stomatal movement. However, at concentrations of less than 100 micromolars it has no effect on stomatal movements (Gomi *et al*., 2016).

Assessment of indicators of chlorophyll fluorescence and flavonoids in stressed plants shows that reducing the the amount of chlorophyll a in the salinity treatment has been the major cause of reduction in excitation capacity of photosystem II, the amount of chlorophyll a in the reaction center is one of the factors determining the efficiency of operation with a excitation capacity of photosystem II (protein D1) (Kaouthet, 2012). The chlorophyll fluorescence rate could show the plant's ability to withstand environmental stresses and the damage that stress applies to the plant. Salinity stress increases Fv, Fm, Fo and reduces Fv / Fm (Table 2). Reports about the effect of salinity on chlorophyll fluorescence are contradictory. In cotton (Brugnoli and Lauteri, 1991), vica faba (Mishra *et al*., 1991) and in Rose (Jimenez *et al*., 1997), it has been reported that changes in Fv / Fm in different levels of salinity are not significant. The researchers concluded that the maximum Fv / Fm can not be raised as an indicator in the salinity stress. In contrast, in the family Brassicaceae (Bongi and Loreto, 1989) and olives (Misra *et al*., 2001), they stated that it is the maximum quantum yield of PSII is and its value for plants that are not under stress conditions, is in a range of 0.85-0.65. If plants are under drought stress, salinity stress, heat and radiation, their rate will decrease (Akram *et al*., 2006). Salinity stress may be the main cause of changes in the ratio Fv / Fm in wheat (Li *et al*., 2008) and Capsicum (Gayyam *et al*., 2007). Epi-brassinolide increases biosynthesis rate of chlorophyll and chlorophyll fluorescence rate which is considered as an operation indicator of PSII (Samira *et al*., 2010). With the help of activating Osmolytes of antioxidant system, this material causes a wisp of ROS caused by environmental stresses and, prevents the decomposition of light reaction center and PSII (Tuna *et al*., 2008). So it can be concluded that Epi-brassinolide can protect the photosynthetic system against salinity stress. The main cause of the stresses are chloroplast membrane and pigment and reduce the amount of PSII and Fv / Fm, but by using Epi-brassinolide, ROS are swept membranes of chloroplasts will be preserved from damage and will therefore the efficiency of PSII is maintained and chlorophyll fluorescence rate returns to its normal state, that is observed in vica faba (Rafael and Esther, 2009) vitis viniferal (Zhizhen *et al*., 2014) It has been reported that, Jasmonic acid improves the chlorophyll fluorescence in Cucumis sativus L. under environmental stresses and changes in the rate and ability of PSII (Stepien and Klobus, 2006). Reduction in the amount of Fv / Fm and PSII efficiency may be due to destruction of pigments that act as antenna and limition of QA and reduced electron transfer from PSII to PSI (Jung, 2004). Program for using of external methyl jasmonate increased Fv / Fm (Figure 2), indicating increased photosynthetic resistance and decrease membrane damage at the plant (Jung, 2004) Under several environmental stresses, increased NPQ along with Photoinactivation can cause damage to the light reaction center and increase F0 at the PS II reaction center (Figure 3) (Jung, 2004). The role of methyl jasmonate on chlorophyll fluorescence recovery

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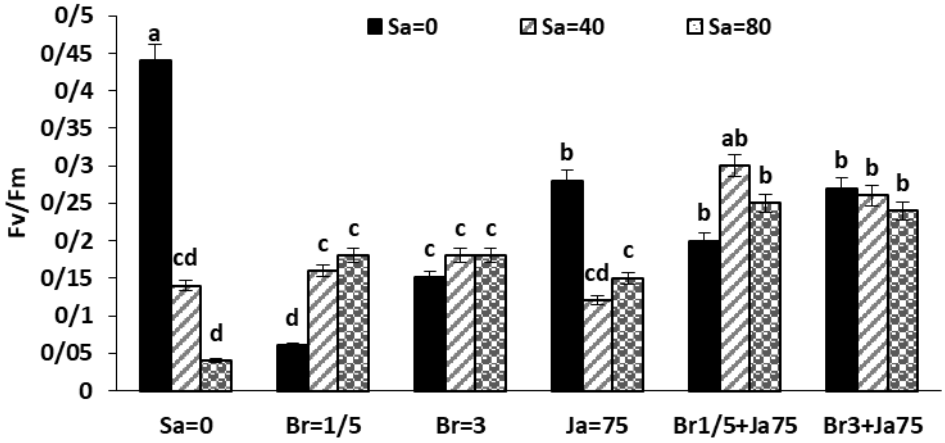

Fig 2. Interaction of salinity stress, epi-brassinolide and methyl Jasmonate treatment on the maximum quantum yield of photosystem II in the dark acclimated (Fv/Fm), Con (Control), Sa (salt), Br (Epi-brassinolide) and Ja (methyl Jasmonate). Results for Mean Mean ± SE by Duncan test (P≤0.05) is shown. Joint letters no significant difference in the level of 5%.

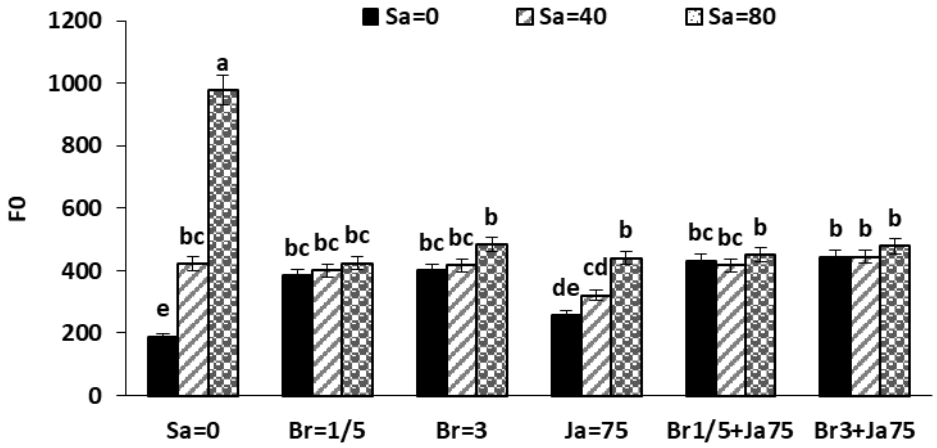

Fig 3. Interaction of salinity stress, epi-brassinolide and methyl Jasmonate treatment on the Initial fluorescence (F0), Con (Control), Sa (salt), Br (Epi-brassinolide) and Ja (methyl Jasmonate). Results for Mean Mean ± SE by Duncan test (P≤0.05) is shown. Joint letters no significant difference in the level of 5%.

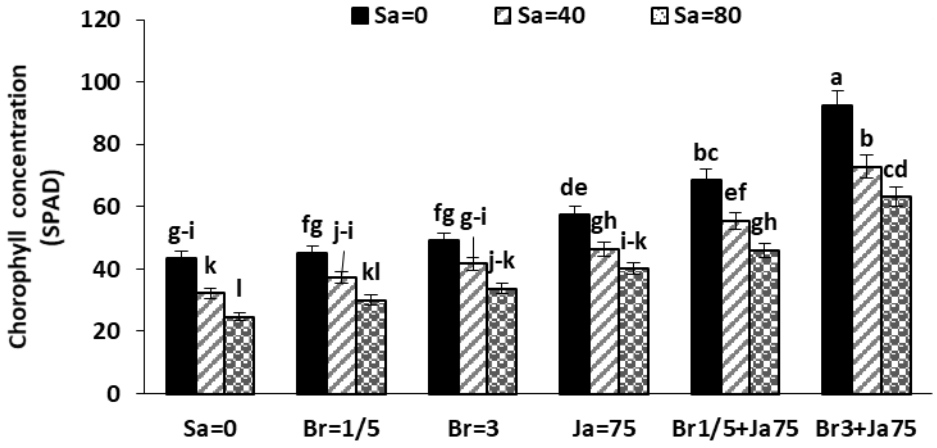

Fig 4. Interaction of salinity stress, epi-brassinolide and methyl Jasmonate treatment on the chlorophyll meter value, Con (Control), Sa (salt), Br (Epi-brassinolide) and Ja (methyl Jasmonate). Results for Mean Mean ± SE by Duncan test (P≤0.05) is shown. Joint letters no significant difference in the level of 5%.

has not yet been studied, however, the role of this substance in protection of pigment systems from oxidative damage and protection of chlorophyll biosynthesis. Thus jasmonate help to increase chlorophyll concentration (Figure 4). Also, methyl jasmonate will be useful in trapping ROS for the health and maintenance of cell membranes, including the membranes of chloroplasts by increasing plant cells

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capacity (Jung, 2004)

Conclusion:

In this study, information on the effects of methyl jasmonate and Epi-brassinolide were presented salinity tolerance in black mustard. Increased maximum fluorescence (Fm), stomatal resistance and reduced transpiration from the leaves are proposed as solutions to deal with salinity. On the other hand, methyl jasmonate and Epi-brassinolide can be considered as an anti-stress combination by increasing stomatal conductance, chlorophyll concentration and Hill reaction rate. Further research in this area can lead to increased recognition of anti-stress effects of methyl jasmonate and Epi-brassinolide and the role of plant hormones in increasing the medicinal properties of plants that are under stress.

Acknowledgements

Hereby we are thanking honorable officials of Islamic Azad University of Damghan branch, honorable officials of Shahr-e-Rey and Damghan laboratory which provided all facilities of the research.

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