Physiological responses of peppermint (*Mentha piperita* L.) to plant growth regulators and salinity stress

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Abstract

Salinity of soil is a major abiotic stress limiting the crop production and growth of peppermint. The aim of study was assessing plant growth regulators' efficacy (salicylic acid (SA), jasmonic acid (JA), brassinosteroids (BRs), and non-application of hormone as control) on physiological traits of peppermint under salinity stress (0, 30, and 60 mM). The experiment was conducted using the completely randomized design (CRD) with three replications at a greenhouse in Varamin, Iran. Results indicated that the salinity stress caused an increase in malondialdehyde and proline contents, activity of superoxide dismutase, and catalase enzymes, and essential oil content while it decreased total chlorophyll content and menthol percentage. In 60 mM salinity, we observed 20.10% decreases of total chlorophyll compared to the control treatment (no salinity). In the interaction effects of salinity and spraying, the highest MDA content was related to the non-application of plant growth regulators under 60 mM salinity (13.8 mol/g FW) while the lowest MDA was recorded under the foliar application of SA in no salinity conditions (5.35 mol/g FW). The highest proline content was observed in the non-application of plant growth regulators under high level of salinity (60 mM) by 7.68 µmol/g FW, which had an increase by 73.5% compared to the control treatment. Finally, the application of growth regulators under salinity stress moderated the negative effects of salinity stress by increasing the synthesis of malondialdehyde, proline, and antioxidant enzymes.

Keywords: Biochemical properties; catalase; malondialdehyde; photosynthetic pigments; superoxide dismutase

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Introduction

Peppermint (*Mentha piperita* L.) belongs to Lamiaceae family. It is an herbal, perennial, and rhizomatous plant. The compounds of peppermint essence are Menthol, Menthone, Menthofuran, Pulgen, Eucalyptol, and Simonton. The combination of these components is a determinant of the essence quality (Afkar, 2015). Peppermint demonstrates antioxidant, antitumor, antimicrobial, antiallergenic, anti-convulsion, digestive, and antiseptic properties (Shahabivand et al., 2018).

Various environmental stresses are the most important factors, which cause difficulties in the production of crops, and the salinity stress is one of the main factors in plant yield decrease, particularly in arid and semi-arid regions (Shaki et

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al., 2020). Sodium chloride is the main source of salinity. As soon as the intracellular changes are recognized, different signaling paths are started to convert the physical stress into an appropriate biochemical response, and each of them causes the expression of a specific group of responsive genes to the stress. Different types of reactive oxygen have an extremely high affinity for reaction with critical biomolecules such as proteins, lipids, and nucleic acids. Damage to the mentioned biomolecules leads to the degradation of proteins, lipids peroxidation, mutation in DNA structure, and finally cell death (Fahad et al., 2012). To decline the damages of reactive oxygen species, plants own antioxidant mechanisms (Alscher et al., 2002). The activity of antioxidant enzymes has a great role in destroying reactive oxygen species. These enzymes enhance the ability of stress resistance in plants and delay the senescence (Ghorbani et al., 2013). Superoxide dismutase (SOD) is a metal enzyme, which is the frontline against the damages of oxygen radicals (Gunes et al., 2007). SOD enzymes destroy produced superoxide radicals by electron transport chain in chloroplasts and mitochondria through producing hydrogen peroxide (H_2O_2). Hydrogen peroxide is destroyed by catalase (CAT) and peroxidase (POX) in different cellular segments (Fahad et al., 2012). Different researchers have addressed the task of this enzyme in cell protection under various stresses. The activity of antioxidant enzymes including SOD exponentially increased in all salt concentrations in two cotton varieties (Gibon et al., 2000). The increase in antioxidant enzyme activities under salinity stress is often associated with the increase in resistance to salinity (Haghshenas and Skandari, 2011).

Salicylic acid (SA) belongs to the secondary compounds which exists naturally and has been detected in more than 34 plant species. Salicylic acid impacts on many cell physiological process (Nasri and Ghaderi, 2014). It is a plant hormone that has a significant role in some plant physiological activities such as respiration control, closure of stomas, seed germination, fruit ripening, glycolysis, flowering, and heat generation (Orabi et al., 2010). It is an important signaling molecule in plant responses against environmental stresses. The treatment of plants with SA improves the effects of salinity and drought in wheat plant (Hussein et al., 2007).

Jasmonates (JA) are the important cellular regulators which participate in plants' different developmental processes including seed germination, root growth, fertility, fruit ripening, and senescence. Furthermore, JA, as a messenger molecule by activating signaling pathways enhances plant tolerance to abiotic stresses such as heavy metals stress (Jaisingh et al., 1993; Afkar, 2015). Some reports indicate this hormone is produced as a result of oxidative stress induced by heavy metals. In plants' reaction against stress, JAs act as the genes encoding of inhibitor proteins such as Threonine, hydroxyl proline, and proline, and totally through activating of plant defense mechanisms help plants in decreasing salt absorption and accumulation (Jaisingh et al., 1993). These signaling molecules are involved in some signal transferring systems and give rise to induction of particular enzyme activities which catalyze biosynthetic reactions associated with the production of defense compounds such as polyphenols, alkaloids, and proteins.

Brassinosteroids (BRs) are a new group of plant growth regulators with the efficacy of growth stimulation. The growth stimulation by BRs results in cell division and elongation. BRs also enhance plants' resistant against environmental abiotic stresses. Tomato and rice plants treated with BRs showed better growth than the controls under cold stress. BRs improved the resistance of corn and cucumber seedlings to the cold stress (Leonardis et al., 2007). In rice, 24-Epibrassinolide has raised resistance against cold stress (Mahmoud and Croteau, 2003). Likewise, it has been reported that the use of BRs increased resistance to drought in sugar beet.

The present study was conducted to survey the effect of spraying plant growth regulators SA, JA, and BRs on physiological characteristics and biochemical properties of peppermint under salinity stress.

Materials and Methods

This experiment was conducted through cultivation of the root grown annual peppermint plants at a greenhouse in Varamin city, Iran, in two consecutive years 2017 and 2018. The geographical position of the experiment locality is

the latitude of 35˚15' N and the longitude of 51˚15' E. This experiment was conducted as the factorial based on a completely randomized design with three replications. The treatments of the experiment included the salinity at three levels: 0, 30, and 60 mM (Ahmad et al., 2009), and the application of the plant hormones was considered at four levels of SA at the concentration of 100 mg/l (Allen, 1995), JA at the concentration of 60 mM (Alscher et al., 2002), BRs at the concentration of 1 mM (Agarwal and Pandey, 2004), and non-application of the hormone as the control. SA, JA, and BRs were provided from Sigma Company agency.

To provide saline soil via the irrigation water, the pure lab sodium chloride with a molecular mass of 58.44 g was used. To calculate the required salt to achieve the desired salinities, the "Salt Calc" software was used. For this, the determination of the soil electrical conductivity was needed; therefore, to determine the initial electrical conductivity of the soil, the extract at the ratio of 1:2 was used and 6 samples of air-dried and sieved soil, each 50 g in mass, were put into 6 Erlenmeyer flasks. Then, 100 ml distilled water was added and they were kept for about 1 hour on the shaker. Next, each Erlenmeyer solution was leached with filter paper and measured by the EC meter device. The average obtained electrical conductivity was 3.93 dS/m.

After cultivation the required salt for every level of salinity was added into each pot soil along with irrigation water at two stages, the first stage 15 days and the second stage 30 days after planting, so that the plants were established in the new environment (Ahmad et al., 2009).

Measurement of SOD enzyme activity

The measurement of SOD enzyme activity was carried out based on Fahad and Bano (2012) method. The reaction solution included a buffer, EDTA, Triton X-100, and Nitro blue tetrazolium, which was added into 2 ml of this solution to react with 100 mM of the plant tissue extract, and its absorbance was measured at the wavelength of 520 nm with the spectrophotometer after a one-minute change at the lab temperature. The enzyme activity was determined according to the standard curve.

Measurement of CAT enzyme activity

Catalase was measured using Paglia (1997) method. In this method, the reaction intensity of hydrogen peroxide elimination is evaluated as a substrate. The background buffer contained 0.17 mM disodium phosphate (pH 7.5) along with 0.15 M EDTA and 0.11 mM Magnesium chloride. One unit of CAT enzyme activity was consider as equivalent to the ratio of hydrogen peroxide conversion in one minute while the first-order reaction proceeded.

Measurement of MDA

For this, the HPLC chromatography method based on the Steven method was used. The extract used for the measurement of 8-oH-dG was based on Thiobarbituric acid with MDA method. To measure the MDA first proteins, we precipitated the use of Trichloroacetic acid solution and separated it by centrifuging. The clear solution supernatant reacted with Thiobarbituric acid at 95 ˚C for 50 min. The product of this reaction was transferred into octadecyl silica column by 12-molar Trichloroacetic acid. After balancing the column, with the mobile phase including washed buffer phosphate, the MDA peak was identified in the spectrophotometer with a visible detector at the wavelength of 532 nm and was measured according to the area under the peak curve. To standardize the pure MDA with different proportions, we washed it and the standard curves were drawn.

Total chlorophyll content

The fresh leaves from each replicate were collected and immediately frozen in liquid nitrogen and stored in the ultra-low freezer at -80 ℃ for physiological studies. Based on the method, 0.25 g of fresh tissue was extracted using 5 ml 80% acetone. Then, the extract was centrifuged at 11000 rpm for 10 min. Besides, the optical density of the extract was measured at the wavelengths of 646.8, 663.2, and 470 nm (Aghighi Shahverdi et al., 2017).

Free proline content

Free proline content was determined according to the method described by Bates et al.

Table 1
Combined variance analysis of salinity stress (S), spraying (F) of JA, SA, and BRs effects on some physiological characteristics of *Mentha piperita* L.

		Mean square (MS)						
SOV	df	T-Chl	MDA	Pro	SOD	CAT	EO	Ment
Year (Y)	1	262.9 ns	0.003 ns	0.0006ns	0.003 ns	0.50 ns	0.00006ns	14.9ns
Rep (Y)	4	10.7	0.007	0.004	0.003	0.94	0.0008	59.7
Salinity stress (S)	2	9.31*	128.1 **	119.3**	309.1 **	9454.8 **	0.027**	2362.7**
Spraying (F)	3	14.8*	34.3 **	3.09**	16.79 **	1174.7 **	0.0049**	197.0*
S×Y	2	3.81ns	0.008 ns	0.0008ns	0.001 ns	0.16 ns	0.00008ns	77.7ns
Y×F	3	19.7ns	0.009 ns	0.004ns	0.004 ns	0.09 ns	0.00007ns	76.4ns
S×F	6	19.94ns	6.33 **	1.28**	4.30 **	274.9 **	0.002**	77.1ns
Y×S×F	6	7.93ns	0.003 ns	0.002ns	0.003 ns	0.37 ns	0.000003ns	46.4ns
Experimental error	44	16.23	0.006	0.009	0.009	0.55	0.00004	58.7
CV (%)	-	8.65	9.98	2.46	7.65	11.17	5.05	11.98

ns, *, and **indicate non-significant and significant at the level of 5 and 1%, respectively; *T-Chl: total chlorophyll; MDA: Malondialdehyde; Pro: proline content; SOD: superoxide dismutase; CAT: catalase activity; EO: essential oil content; Ment: Mentol content*

(1973). Approximately 0.25 g of fresh seedling material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Finally, 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer.

Essential oil and menthol content

Essential oil samples were analyzed using GC–MS (GC-2010 SHIMADZU), according to the method described in detail for the type, column, etc. by Fattahi et al. (2016). Peppermint leaves were collected and hydrodistilled using a Clevenger apparatus. Gas chromatography/mass spectrometry (GCMS) analysis was equipped with a split–splitless injector (split ratio; 30:1), scan time 1s, ionization energy 70 eV, and mass range of 40-300 amu. a column (60 m × 0.25 mm i.d., film thickness 0.25 m); oven temperature was 60-230 ℃ at a rate of 7 ℃/min and transfer line temperature 260 ℃. The carrier gas was helium, with a linear velocity of 31.5 cm/s. The oils were diluted in dichloromethane (2 µl of oil in the 2 ml solvent), the next 2µl oil of each treatment was injected into GC/MS manually. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes C8-C22 on the HB-5 column. The essential oil constituents were determined by comparing their GC retention indices, mass spectra with data published in the literature by Adams. Compounds were further identified using their mass spectra data compared with National Institute of Standards and Technology mass spectra library data provided by the software of the GC-MS system. Menthol content was shown as a relative percentage of the total oil.

Statistical Analysis

After checking the data distribution normality assumption (Kolmogorov-Smirnov and Shapiro-Wilk test), the studied traits were statistically analyzed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2). The differences among means were separated using the Duncan test at 0.05 statistical probability level and the graphs were drawn by MS–Excel.

Results

Total Chlorophyll content

The effects of salinity stress and spraying of growth promoting regulators were significant on total chlorophyll content ($p \leq 0.05$) (Table 1). Salinity stress decreased the total chlorophyll content. In 60 mM salinity, we observed 20.10% decrease compared to the control treatment (free salinity). On the other hand, significant differences were observed between 0 and 30 mM levels in terms of chlorophyll content (Table 2). Foliar

Table 2
The effects of salinity stress and spraying of JA, SA, and BRs effects on some physiological characteristics and biochemical properties of *Mentha piperita* L.

Treatments	T-Chl (mg/g)	MDA M/g FW)	Pro (µM/gFW)	SOD (U/mg protein)	CAT (U/mg protein.min)	EOC (%)	Ment (%)
				Experiment years			
First	4.46 a	7.87 a	3.96 a	5.98 a	63.30 a	0.20 a	41.01 a
Second	4.46 a	7.88 a	3.96 a	5.99 a	63.47 a	0.20 a	41.13 a
				Salinity stress (mM)			
Control	4.467 a	5.60 c	1.91 c	2.61 c	42.5 c	0.16 c	42.18 a
30	4.034 a	7.79 b	3.64 b	5.59 b	65.6 b	0.21 b	38.24 b
60	3.569 b	10.22 a	6.33 a	9.76 a	82.0 a	0.22 a	30.39 c
				Growth regulators spraying			
Control	4.469 c	9.93 a	3.71 c	4.57 d	51.5 d	0.17 d	42.02 c
Jasmonic acid (JA)	4.514 c	7.05 d	4.34 b	6.70 a	69.6 a	0.21 a	44.18 b
Salicylic acid (SA)	4.83 a	7.11 c	4.58 a	6.46 b	67.0 b	0.207 b	46.03 a
Brassinosteroid (BRs)	4.482 b	7.39 b	4.41 b	6.22 c	65.3 c	0.204 c	43.01 c

The means with common letters in each treatment column do not have any statistical significant difference at 5% level of probability based on the Duncan's multiple test.

application of plant growth regulators in comparison with non-foliar treatment led to an increase in chlorophyll content, the highest average of this trait being observed in SA treatment (4.83 mg/g FW) which had 7.47% increase compared to the control treatment (Table 2).

MDA content

With regard to the results of variance analysis, the year effect on MDA content was not significant in peppermint (Table 1). Application of salinity stress was significant at 1% level of probability on MDA content in peppermint. So, with an increase in the salinity rate, the MDA content enhanced as well. The minimum MDA content was associated with the control treatment by 5.60 M/g FW and the maximum was related to the salinity stress of 60 mM by 10.22 M/g FW (Table 2). In the interaction effects of salinity and spraying, the highest MDA content was related to the non-application of plant growth regulators under 60 mM salinity (13.8 M/g FW) and the lowest was the foliar application of SA under no salinity conditions (5.35 M/g FW) (Fig. I).

Proline content

As shown in Table 1, the effects of salinity stress, spraying plant growth regulators, and interaction of salinity × spraying were significant on proline content ($p \leq 0.01$). Salinity stress and spraying of plant growth regulators increased the proline content (Table 2). The highest proline content was observed in the non-application of plant growth regulators under a high level of salinity (60 mM) by 7.68 µM/g FW, which had an increase of 73.5% compared to the control. Application of JA under 0 mM salinity resulted in the lowest proline content (1.82 µM/g) (Fig. II).

SOD enzyme activity

With regard to the results of variance analysis (Table 1), the year effect on SOD activity was not significant. Application of salinity stress was significant at 1% level of probability on SOD activity so that with an increase in the salinity rate, the SOD activity enhanced as well. Therefore, the minimum SOD activity was associated with the control treatment (without salinity stress) by 2.61 U/mg protein while the maximum was related to the salinity stress of 60 mM by 9.76 U/mg protein (Table 2). Likewise, application of growth regulators had a significant effect on SOD activity at 1% level of probability, increasing its activity (Table 2). Considering the interactions of salinity and plant growth regulators spraying, the highest SOD activity (11.2 U/mg protein) was observed in

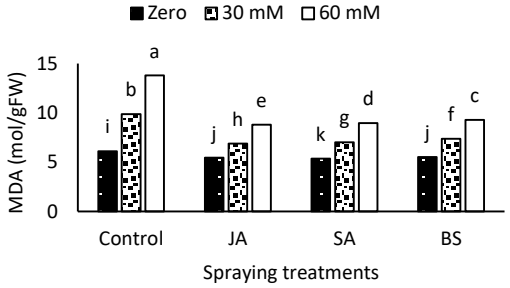

Fig. I. Interaction effects of the plant growth regulators spraying and salinity stress on MDA content

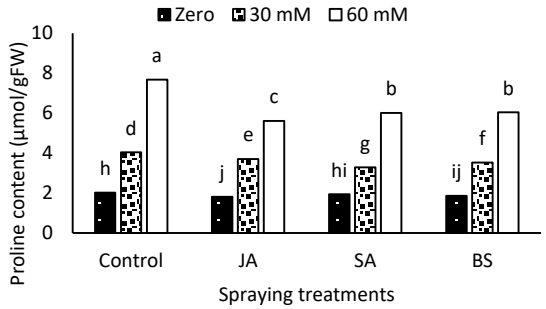

Fig. II. Interaction effects of the plant growth regulators spraying and salinity stress on proline content

the JA spraying under high level of salinity while non-application of plant growth regulators under non-stress conditions resulted in the lowest enzyme activity (2.43 U/mg protein) (Fig. III).

CAT enzyme activity

As shown in Table 1, the year effect on CAT enzyme activity was not significant. Application of salinity stress was significant at 1% probability level on CAT enzyme activity, so that with an increase in the salinity rate, the CAT enzyme activity also increased. In 60 mM, CAT activity showed 48.17% increase compared to the treatment without salinity (Table 2). Like SOD enzyme activity, the highest CAT activity was related to JA application under 60 mM salinity (92.5 U/mg protein.min) and the lowest (40.33 U/mg protein.min) was control treatment (non-application of regulators and without salinity) (Fig. IV).

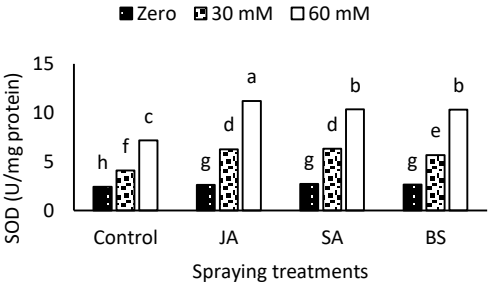

Fig. III. Interaction effects of the plant growth regulators spraying and salinity stress on SOD enzyme activity

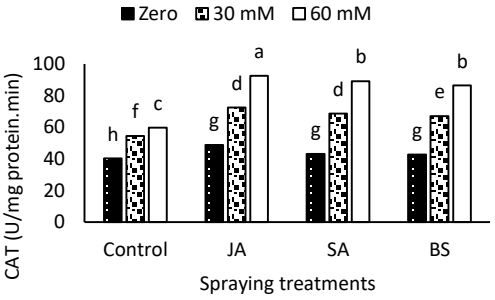

Fig. IV. Interaction effects of the plant growth regulators spraying and salinity stress on CAT enzyme activity

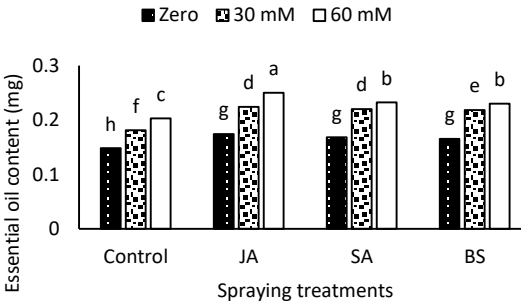

Fig. V. Interaction effects of the plant growth regulators spraying and salinity stress on essential oil content

Essential oil content

As shown in Table 1, effects of salinity, sprayingplant growth regulators, and interactions of salinity and spraying were significant on essential oil content ($p \leq 0.01$). Results indicated that the salinity stress and plant growth regulators spraying increased the essential oil content (Table 2). The highest essential oil content was observed under JA application under 60 mM salinity (0.25 mg) while the lowest (0.148 mg) belonged to non-application of plant growth regulators and no salinity stress conditions (Fig. V).

Menthol content

Combined variance analysis results showed the effects of salinity and spraying plant growth regulators were significant on menthol content at p≤0.01 and p≤0.05, respectively (Table 1). Salinity stress decreased menthol content while the spraying treatments increased menthol content. The highest menthol content was related to salinity stress free condition (42.18%) and SA spraying (46.03%) (Table 2).

Discussion

In the present study, total chlorophyll and menthol contents were inhibited by salinity stress while the salinity increased synthesis of MDA, proline content, the activity of SOD and CAT enzymes, and essential oil contents. The decrease in growth and yield, also due to treatment with salinity, could be due to the negative effect of salinity on photosynthetic pigments, changes in enzyme activity, and also the growth regulating hormones, which can lead to inhibition of the growth (Abdol-Qados, 2011). One of the earliest consequences of salinity stress is the promotion of an osmotic effect in the soil, limiting water uptake by the plant (Aghighi Shahverdi et al., 2017).

With an increase in the irrigation water salinity, the rate of fats peroxidation and after that MDA content increased. The research findings suggest that under drought and salinity stress conditions, peroxidation of membrane lipids rose and this led to the production of aldehydes such as MDA and other products like ethylene. It was also reported that under stress conditions, MDA increases and causes a decrease in the plant resistance index and cell membrane concentration. In this regard, the results of the present research are consistent with the those of of Sairam et al. (2001). The efficacy of spraying with the growth regulators was significant at 0.01 level of probability on MDA content as well. The growth regulators reduced the content of MDA. Treating with jasmine acid caused the minimum MDA production and the control treatment (non-application of growth regulators) had the maximum MDA production.

The peppermint treatment with JA caused the maximum SOD activity that is consistent with the research results of Yusuf et al. (2008), Fahad et al. (2012), and Gunes et al. (2007). Also, the treatment with JA and BRs resulted in significant rises of SOD in peppermint, which is consistent with the research results of Afzali et al. (2007) and Jaleel et al. (2008).

The minimum CAT enzyme activity was associated with the control treatment (without salinity stress) while the maximum amount was related to the salinity stress of 60 mM that is consistent with the research results of Sairam et al. (2002) and Husein et al. (2007). CAT breaks down H_2O_2 into water and oxygen. It is responsible for the degradation of H_2O_2 produced in peroxisomes during photorespiration, or H_2O_2 produced at beta-oxidation in glyoxysome, or H_2O_2 produced by SOD (Agarwal et al., 2004).

Application of growth regulators increased CAT enzyme activity under salinity stress. The results indicated that the treatment of peppermint with JA and SA caused the maximum and minimum CAT enzyme activities, respectively in the control treatment which is consistent with the research results of Yusuf et al. (2008). Through the increase in the irrigation water salinity, fats peroxidation rate, and after that MDA content increased. It has also been reported that under stress conditions, MDA increased and resulted in the decrease in the resistance index and cell membrane concentrationwhich is consistent with the research results of Sairam et al. (2002) and Ahmad et al. (2009).

To cope with the oxidative stress, plants own a very effective defense system, which can eradicate or neutralize free radicals. This defense system comprises enzymatic and non-enzymatic defense systems (Ozdemir et al., 2004). These defense system enzymes include ascorbate peroxidase (APX), CAT, SOD, glutathione reductase (GR), and dehydroascorbate reductase (DHAR). To cope with oxidative stress, a non-enzymatic defense system embraces ascorbic, Alpha-Tocopherol, and vitamins. It has been reported that the alteration in the activity of antioxidant enzymes in response to salinity varies in resistant and susceptible varieties (Rios-Gonzalez et al., 2002). Researchers have suggested that a strong relationship exists between the tolerance of oxidative stresses, which are created by the environmental stresses, and an

increase in the concentration of antioxidant enzymes in photosynthetic plants (Hussein et al., 2007). The findings from the assessment of salinity stress reveal the significant difference between various genotypes of wheat in terms of leaf relative water content, chlorophyll content, membrane resistance index, as well as the activity rate of hydrogen peroxide acrobat, SOD, peroxide, and GR. Also, the less decrease in water relative content, chlorophyll, and membrane resistance index in these varieties, in response to salinity stress, have been attributed to SOD, APX, and GR, resistance to higher activity (Yusuf et al., 2008). The increase in free radicals such as Superoxide occurs while a plant confronts with intense drought and salinity stresses. In this regard, the next plant reaction is the synthesis and more activity of SOD enzyme for further neutralization of Superoxide destructive anion. The product of this reaction is hydrogen peroxide. Research has rshown that peroxide hydrogen content depends on the severity of the drought stress plant encounters (Hussein et al., 2007).

The studies show that the species adapted to arid and salty regions start increasing the activity of antioxidant enzymes such as peroxide, SOD, and CAT under the condition of drought and salinity stresses in order to neutralize the produced free radicals (Hussein et al., 2007). It has also been reported that SA has a protective responsibility in plant salinity resistance. The use of SA has increased the activity of antioxidant enzymes such as CAT, POX, and SOD (Koocheki et al., 2008), which is consistent with the research results of Sairam et al. (2001, 2002); and Hussein et al. (2007). Cell division and elongation decreased under salinity stress. It sounds that SA along with other substances like auxin controls cellular division and elongation (Mittler, 2002).

The results of the experiment indicated that there is a positive and strong correlation between the tolerance to oxidative stresses, which are generated because of the environmental stresses, and the increase of antioxidant enzymes concentration (Yusuf et al., 2008). In this regard, investigations have demonstrated that in severe stresses the concentration of antioxidant enzymes has doubled, and consequently the plant resistant to oxidative stresses has risen. So, the mechanisms in plants that result in the decline of oxidative stress can have a great impact on plant adaptation to stressful environments (Hussein et al., 2007). Studies suggest that the species adapted to arid regions start increasing the activity of the antioxidant enzyme such as peroxide, SOD, and CAT under the condition of drought stress to neutralize the produced free radicals (Hussein et al., 2007). It has also been reported that SA has a protective role in plants salinity resistance. There is some evidence that plant photosynthesis intensively declines due to the synthesis diminution or degradation of plant pigments including chlorophyll because of salinity (Ozdemir et al., 2004). With regard to the results of the present research as well as the experiments of other researchers, it can be argued that once peppermint plant is exposed to salinity stress condition, it tries to mitigate the negative effects of salinity by activating antioxidant enzymes biosynthesis paths and the enhancement of these enzymes activity (Andre Dias et al., 2006). The deactivation of reactive oxygen species is accomplished through a complicated process that costs a lot of energy for the plant, but it is one of the best methods for the protection of cell different segments structure including plasma membrane structure and cells life protection under salinity stress (Bates et al., 1973). Through the rise of the salinity stress, the production rate of MDA, and the activity of CAT and SOD enzymes increases.

According to the findings, salinity stress increases the rate of biomarkers in cell degradation, including MDA in plants such as peppermint. Also, with increasing salinity stress, the activity of antioxidant enzymes such as CAT and SOD and also proline content increased. Also, plant growth regulators such as SA, JA, and BRs, with an effect on different biochemical processes within plant tissues, have mitigated destructive effects of salinity stress in the plant. The application of the plant growth regulators led to the activation and biosynthesis of antioxidant enzymes such as SOD and CAT under the salinity stress in peppermint plants. These plant growth regulators decreased the amount of the produced degradation biomarkers such as MDA, which is an indicator of the salinity stress condition modification due to the activation of the plant resistance biosynthesis paths in the salinity stress

conditions. JA, SA, and BRs cause the modification of the salinity stress condition by inducing the antioxidant enzymes biosynthesis paths and through the prevention of CAT and SOD.

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