The effects of methyl jasmonate and salinity on germination and seedling growth in *Ocimum basilicum* L.

Shekoofeh Enteshari* and Tahereh Jafari

Biology Department, Payam Noor University, I.R. Iran

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Abstract

About 10 percen of lands around the world under crops planting are affected by salinity. Therefore, comprehensive researches have been conducted to minimize the harmful effects of salinity in agriculture. The purpose of this study is examination of the Mmethyl jasmonate effect on reducing the effects of salinity in germination and vegetative stages of the basil plant.For the methyl jasmonate treatment, seeds were soaked in methyl jasmonate (0,0/1 and 0/01 µM) and then seeds were placed on filter papers wet using NaCl (50, 100, 200 mM). Then, the morphological parameters examined. The results indicated that high concentration of salinity cause to decrease in germination percentage and Methyl jasmonate cause increase in germination percentage, and this indicated the positive effect of methyl jasmonate on improvement of stress conditions in plant. Furthermore, salinity increases the growth of root and shoot, and methyl jasmonate offsets this reduction.

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Introduction

About 10 percent of lands around the world under crops planting are affected by salinity . Plant growth is limited by unfavorable environmental conditions and salinity stress is discussed as one of the biggest problems in agriculture around the world. (Kong et al., 2005).

Salinity causes imbalance in ions of plant cells, creation of oxidative stress and in consequence, decrease in the growth of plant (Kepczynski and Bialecha, 1994). Salinity inhibits water uptake by plant and decreases soil water

potential and puts the plants under drought stress too (Hagemeyer, 1996). Entrance of ions from the membrane into the internal parts causes balance alteration of plant solution and hormones, and by this, the growth of root and shoot are affected (Lerner et al., 1999).

The effect of salinity on leaf number, leaf area, and dry weight of shoot and root of colza plant has been reported. The growth of rootlet and shootlet and percentage of germination decreases by salinity and this reduction rises by increasing the level of salinity (Steppuhn et al, 2001).

The plant can react to salinity by some changes and offsets in cell process and morphological structure in order to decrease ion toxicities and keep balance between production and destruction of oxygen radicals (Kepczynski

and Bialecha, 1994). These kind of morphological changes in plants adjust pace and amount of missed water by plant, indirectly increase water potential and dilution of solute concentration in cell water, and offset adverse effects of salinity (Jain et al., 2001).

The first line of defense against excessive sodium entry into the plant is cell plasma membrane of root. After altering the ionic composition, water and solute get into the root center by symplast. Through the xylem, parenchymal cells of vascular elements flow to transfer to the aerial. Parenchymal cells are also able to transport salt to the phloem and decrease salt concentration in xylem and prevent it from getting into leaves. Another physiological reaction to salinity is xylem loading by star shaped cells. These cells transfer K^+ and Na^+ ions to xylem which is controlled genetically (Gibberd et al., 2002).

Assignment of salt to the cell vacuole is another physiological mechanism to reduce the level of cytoplasmic ions. If Na^+ and Cl^- are accumulated within the vacuole, K^+ and soluble material must accumulate in the cell cytoplasm to balance the osmotic pressure of ions into the vacuole. Accumulation of Na^+ and Cl^- in vacuoles causes a high osmotic potential for water absorption and cell turgor supply which is one of the effective factors in the growth of plant cell (Munns, 2002).

Jasmonates are one of the newest plant growth regulators which cause decrease in damages due to environmental stresses in plant (Wang, 1999). Jasmonic methyl ester acid was discovered first as a matter of fragment from oil of *Jasmonium grandiflorum* (Sembdner and Parhtier, 1993), and jasmonic acid was derived from fungal culture in 1971. These compounds have also been observed in plants e.g., in tea, rosemary and mint and some fungi (Creelman and mullet, 1995). Pericarps, reproductive structure particularly ovarian and the elongation zone of root and stem have a high amount of jasmonate. Jasmonates exist in 150 type including ferns, mosses and fungi and 206 plant species have been identified to contain these compounds (Creelman and Mullet, 1997).

Jasmonic acid is produced as a signaling molecule in response to external stimulators such as wounding, mechanical force, pathogen attack and osmotic stress (Molina et al., 2002). For example, at the wound site, cysteine with 18 amino acid transports by vessels to different parts of plant and by poly-peptides hydrolysis of the wound site caused induction of jasmonic acid. Genes responsible for the Jasmonate acid are expressed in apical part of plant. Increase in jasmonic acid controls compound biosynthesis such as proline and putrescine in environmental stresses and induces the genes of these compounds in plants. In the fungal pathogen attack, jasmonate activities encode proteases, and reduced the damages. Furthermore, it adjusts proteins of wall like PRP which may be necessary for barrier synthesis against infection (Gao et al., 2004).

In soybean, at seedling stage, the level of jasmonate has mostly been observed in hypocotyls, the region of cell division, root and old leaves. In mature leaves of soybean, it is observed in mesophilic cells and vascular sheath surrounding the vein, and the small amount in epidermal cells. A small amount of jasmonate was observed in palisade parenchyma cells, and it has been reported that the low level of jasmonate in these cells is due to division of parenchyma cells or low sensitivity of these cells to jasmonate (Creelman, Mullet. 1997). In rice root under salinity stress, the amount of methyl jasmonate increased and harmful effects of salinity on photosynthesis and growth decreased (Wang et al., 2001).

Jasmonate is involved in physiological processes including seed germination as a plant growth regulator. In some cases it was reported that jasmonates inhibited germination of seeds without dormancy and stimulated germination of dormant seeds. In apple, jasmonate stimulates germination of dormant embryo. In dormant seeds, jasmonate may stimulate germination of seed by decrease in sensitivity to abscisic acid. One research also showed that methyl jasmonate at the concentration of 10-3 M prevents from germination of pigweed seeds (Kepczynski and kepczynska, 1999). On the other hand, germination in sunflower, wheat and oat was inhibit by jasmonate (Nojavan and Ishizawa, 1998)

The present work was carried out to investigate the effect of different concentrations of metyl jasmonat and NaCl on germination and seedling growth of *Ocimum basilicum* L**.**

Materials and Methods

Seeds of green basil plant were prepared from agricultural research center in Isfahan, Iran. After separating healthy and uniform seeds, 25 seeds were placed in Petri dishes containing filter paper and for each treatment 4 Petri dishes were considered as the 4 replicates.

In order to give salinity treatment, sodium chloride solution with concentrations of 0, 50, 100 and 200 mM were added to Petri dishes containing the seeds. For methyl jasmonate treatment, seeds were soaked at concentrations of 0, 0.1 and 0.01 µM for 6 hours and then were transferred to Petri dishes. Also for the combined treatments, after soaking the seeds at the mentioned methyl jasmonate concentrations, they were transferred to Petri dishes and irrigated by sodium chloride solution at the mentioned concentrations. Then, the Petri dishes were placed in a germinator (model KH RH 1, Iran) at the temperature of 16 °C under light conditions 16:8.

Germinated seeds in Petri dishes were counted after 24, 36 and 48 and 60 and 72 hours after cultivation. Percentage of germination and the required times for germination were calculated using the following formulas (Torabi et al 2012):

Percentage of germination = number of germinated seed / total number of seeds ×100

Average time for germination = $N_1 T_1 + N_2 T_2 + N_3 T_3$ / N where N_1, N_2 ,… N_n show number of germinated seeds in the first, second and n_{th} day, respectively. T_1, T_2, … T_n show the count of first, second, … n_{th} day, and N shows the total number of germinated seeds. The shootlet and rootlet length and wet and dry weight were then measured. Statistical analysis was done by SAS software with 4 replicates and mean values and significance were determined by Duncans multiple range test at 5% probability level.

Results

Percentage of germination

Interaction of effects of methyl jasmonate and salinity on germination percentage indicated that compound treatment of methyl jasmonate and salinity at concentration of 200 mM decreased this parameter significantly (Fig. I. b) (P≤0.05). Also, salinity levels had no significant effect on the average time for germination. The compound treatment of methyl jasmonate (0.1 µM) and salinity at concentration of 200 mM increased this parameter as compared with the control salinity (Fig. I. a) (P≤0.05).
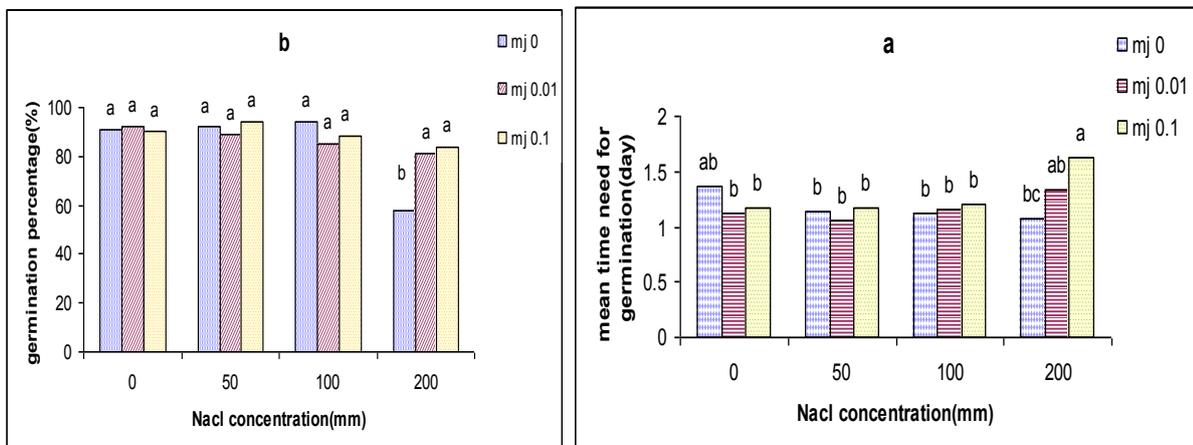
Rootlet length

Fig. I. Interaction of effects of methyl jasmonate and salinity on the average time for germination and percentage of germination; averages with similar letters are in one category and are not significantly different at 5%.

Rootlet length was decreased by increasing level of salinity, so that the lowest level of this parameter was observed at 200 mM of salinity as compared to control plants. Use of Methyl jasmonate in treatment of 0.1 and 0.01 µM and salinity concentration of 200 mM caused a significant decrease in rootlet length (Fig. II. b) (P ≤ 0.05).

Shootlet length

Increase in salinity levels of 100 and 200 mM caused significant decrease in shootlet length. The shortest length of shootlet goes to salinity level of 200 mM. Methyl jasmonate at concentration of 0.1 µM and salinity level of 100 mM caused significant adjustment in decrement of shootlet length caused by salinity (Fig. II. a) (P≤0.05).

Seedling fresh weight

Salinity treatment at concentrations of 100 and 200 mM caused significant decrease in fresh weight of seedlings. The compound treatment of methyl jasmonate and salinity at salinity level of 200 mM indicated a significant increase in fresh weight as compared with salinity treatment without methyl jasmonate (Fig. III. a) (P ≤ 0.05).
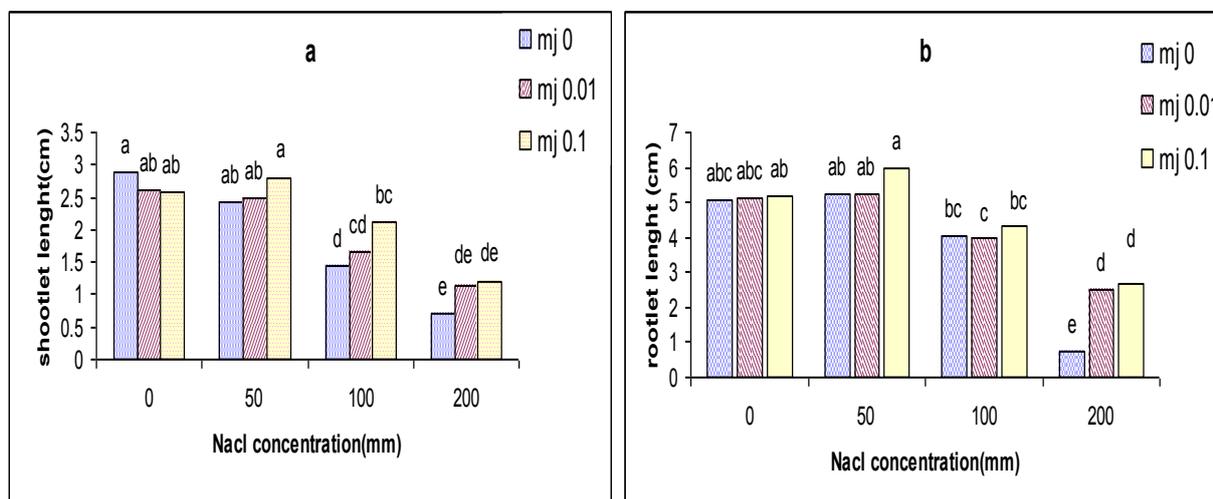
Seedling dry weight

Fig. II. Interaction of effects of methyl jasmonate and salinity on rootlet and shootlet length; averages with similar letters are in one category and are not significantly different at I5%.
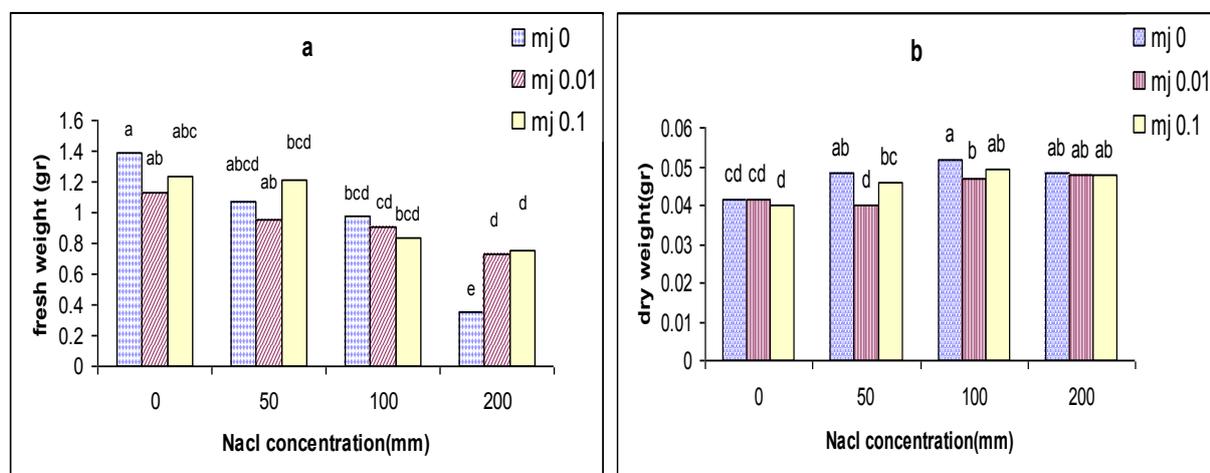

Fig. III. Interaction of effects of methyl jasmonate and salinity on fresh and dry weight of seedling; averages with similar letters are in one category and are not significantly different at level of 5%.

Different salinity concentrations caused significant increase in dry weight of seedlings as compared to control plants. Methyl jasmonate at concentration of 0.01 µM and salinity levels of 50 and 100 mM caused significant decrease in this parameter as compared to salinity level of 50 mM without methyl jasmonate (Fig. II. b) (P ≤ 0.05).

Discussion

The most important vegetative stage of a plant under salinity stress is the stage of germination. High concentration of salt reduces the germination percentage which may be probably due to the increased osmotic pressure or sodium ions within the seed. Sodium ion could be a toxicity factor resulting in reduced seed germination (Ozkan et al., 2005).

Absorption of sodium and chloride ions causes impaired water uptake and secretion of hormones, and modifies the activity of enzymes, such as Malate dehydrogenase and the glucose 6 – phosphate dehydrogenase enzymes which have a major role in grain growth.

Plants resistant to salinity transport less sodium and chloride to the leaves and are able to separate these ions in vacuoles to prevent their accumulation in the cytoplasm or cell wall, and prevent the ionic toxicity of them (Munns, 2002). Furthermore, increased salinity level causes a decrease in germination percentage and increase in average time for germination. Increased resistance to salt stress depends on the plant species and the growth stage. For example, rice is a resistant plant to salinity at germination stage but it is a sensitive plant to salinity at the early stages of growth and flowering. Sugar beet, maize, and sunflower are sensitive to salinity at the germination stage, but later in the growth their sensitivity to stress becomes less. In colza types, germination stage is sensitive to salinity and causes production of abnormal seedlings (Zheng et al., 2001). Kav and his collaborators (2004) exposed cultivar colza and transgenic colza to salinity stress, and observed that germination increases in cultivar type by increasing salinity, but transgenic plants are resistant to salinity.

In this study, high concentrations of sodium chloride caused a decrease in germination percentage due to slowing water absorption by seeds. Toxicity of absorbed sodium and chloride by seed causes modification in enzymes activity and hormones secretion, and can decrease germination percentage. Also methyl jasmonate in the study increased germination percentage at salinity level of 200 mM which shows its positive role in improving the conditions of stress in plant.

Although the effect of methyl jasmonate on the stimulation or inhibition of germination depends on the concentration of methyl jasmonate, Jasik and Klerk (2006) indicated that methyl jasmonate increases germination percentage. Methyl jasmonate and jasmonic acid have inhibitory role in dormant seeds and stimulatory role in seeds without dormant period. It is reported that this composition has an effect on a kind of oak (*Quercus robur*) and methyl jasmonate concentration increases before drying and loss of seed viability. Increased methyl jasmonate caused membrane peroxidation and damage to the cell membrane (Creelman and Mullet, 1997).

In eggplant, sodium chloride concentration of higher than 50 µM delayed germination, but the final germination percentage did not fall, as germination percentage significantly decreased only at high salinity (100-150 µM (Dantas et al., 2005). By increasing salinity up to 100-150 mM, rootlet length was reduced 36 and 74 percent. Seedling appearance also delayed at high levels of salinity (Cheong and Choi, 2003). Seedling stage in eggplant is more sensitive than germination stage.

Similar results are observed in pepper (Chartzoulakis and Klapaki 2000). Reports have shown that ACC levels in treated cotyledons with jasmonate decreased. Thus, methyl jasmonate decreased ethylene synthesis by affecting ACC and ACO, causing a decrease in germination.

In pigweed (Kepczynski and Bialecka, 1994) and sunflower (Corbineau et al., 1988), jasmonate decreased germination. Acid jasmonate also stimulated germination in apple and caused activation of alkaline lipase. Activation of lipase stimulates fats and supplies required sugars for the germination stage. Inhibition of lipooxygenase activity, which is one

of the key enzymes in biosynthesis pathway of jasmonates, decreases germination (Creelman and Mullet, 1997). Furthermore, by blocking other intermediates of biosynthesis pathway of jasmonates, germination would be inhibited.

The growth of plants is a result of cell division and irreversible increase in cell volume. Non-environmental stresses affect cell division and decrease the growth. In this research, methyl jasmonate compensated decrease in germination only at high salinity concentrations. Salinity concentrations of 50 and 100 μM had no significant effect on percentage of germination and average time for germination. This was probably due to the rapid germination of green basil seeds.

Salinity causes ion imbalance in plant cells, causing oxidative stress and decrease in the growth of plant (Kepczynski and Bialecka, 1994). Plants through a series of changes or adjustments in the cellular process and morphological structures can respond to salinity stress in order to reduce ion toxicity and maintain the balance between production and loss of oxygen radicals (Kepczynski and Bialecka, 1994). Under salinity, osmotic pressure decreases and decrease in turgescence is an important factor in the inhibition of growth, as turgor pressure stops cell division and cell elongation. Entrance of salt from root membrane to the inner parts causes alteration in the balance of plant solutions and hormones, and affects the growth of roots and shoots (Lerner et al., 1999). Researches on colza plant demonstrated that following the salinity stress, chlorophyll concentration, root growth (Kaya, et al., 2001), shoot and root dry weight (Steppuhn et al., 2001) decreased in plants. It is reported that salinity decreases the growth of root and shoot, and this reduction will increase by increasing salinity. Decrease in length can be due to decrease in turgor and cell elongation. Similar results have been observed in salinity treatment of two sensitive and resistant types of rice (Kang et al., 2005) and cowpea (Dantas et al., 2005).

It is reported that jasmonate prevents coleoptiles elongation through inhibition of glucose formation in polysaccharides of the two walls (Creelman and Mullet, 1997). Obtained results conformed the decrease in the growth of shootlet by methyl jasmonate in seedling of rice (Yeh et al., 1995) in pharbitis nil and pepper. In the experiment conducted on pharbitis nil, jasmonate treatment increased root length. This was attributed to recreation of the root meristem (Maciejewska and Kopcewicz, 2002).

In this research, high levels of sodium chloride decreased shootlet and rootlet length. 0.01 μM of methyl jasmonate and 100 mM of salinity decreased shootlet length while 200 mM of methyl jasmonate and salinity decreased rootlet length. Thus, the stimulatory effect on rootlet and shootlet growth can only be observed under sodium chloride inhibitory conditions. 0.01 μM of methyl jasmonate caused significant decrease in dry weight of seedlings at salinity levels of 50 and 100 μM which might be due to inhibitory effect of methyl jasmonate on photosynthesis. On the other hand, all sodium chloride concentrations in the study caused a significant increase in dry weight and sodium chloride concentrations of 100 and 200 mM decreased fresh weight significantly. Therefore, we can conclude that high levels of sodium chloride due to changes in water potential make it difficult for water absorption while the amount of dry matter in plant increases.

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