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Application of exogenous Salicylic acid and Malic acid in salinity condition

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Abstract

This investigation was carried out to observe effect of organic acids priming on resistance to salinity. Dry weight (DW) and Ka $^{+}/Na^{+}$ ratio of root and shoot (g plant^{-1}), percentage water content (WC %), leaf area (cm^{2}), chlorophyll a+b and percentage relative water content (RWC %), MDA content and SOD activity were measured. The results of this experiment indicated which application of organic acids were effective in resistance to salinity because of improvement in measured parameters so that the highest of MDA content and the lowest of Dry weight (DW) and Ka $^{+}/Na^{+}$ ratio of root and shoot (g plant^{-1}), percentage water content (WC %), leaf area (cm^{2}), chlorophyll a+b and percentage relative water content (RWC %) and SOD activity were observed in NaCl (150 mM) but application of organic acids caused amelioration of salt stress effects.

Key words: Malic acid, malondialdehyde, Salicylic acid, salt stress, superoxide dismutase, *Vicia faba*

Abbreviations: MA, malic acid; SA, Salicylic acid; MDA, malondialdehyde; ROS, reactive oxygene species; SOD, superoxide dismutase; DW, Dry weight; WC, water content; RWC%, percentage relative water content.

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1.0 Introduction

Seed priming is one of the physiological methods for improving of seed performance and providing faster and synchronized germination [1] in fact, in the seed priming, seeds expose to low water potentials which it is caused restriction of germination and physiological and biochemical changes of pregerminative [2]. When seeds are in the primed state, important pre-germinative steps such as DNA and RNA synthesis are accomplished in the seed, hence the seeds are physiologically close to germination and have fewer steps to complete than unprimed seed in order to accomplish germination. [3,4]. According to the report of Nagar, et al. [5] hydro- priming is effective on field emergence, its rate and early seedling growth of maize *(Zea mays* L.) crop. Salinity is caused restriction of crop productivity, therefore for improving of deleterious effects of it on plants, strategies has been used, one of the strategies is application of salicylic acid. [6]. Salicylic acid (SA) is an endogenous growth regulator of Phenolic nature influencing a range of diverse processes in plants including seed germination [7], ion uptake and transport [8] also is considered as a hormone-like substance, which plays an important role in regulating a number of physiological processes and provide protection against biotic and abiotic stresses in plant. The protective function of SA includes the regulation of ROS and antioxidant enzymes [9,10]. Malate is the organic acid with a diverse role in plant from osmotic balance of vacuole [11], central role in *pH* regulation [12], and as an energy source for plant mitochondria [13]. Raskin [14] reported important role of SA is in growth and development processes of plants. Seed priming with salicylic acid maintenance of tissue water contents and reduced membrane permeability [15]. Several studies have shown that salicylic acid is an essential component in the plant response to adverse environmental conditions such as salinity and making resistance to plant against to this stress [16, 17]. By means of stimulus a secondary oxidative stress that leads to an increase of reactive oxygen species (ROS). At low levels, ROS act as signal molecules that induce adaptive responses [18] also. Gunes et al., [19] know SA as a signaling molecule responsible for inducing abiotic stress tolerance in plants. Ben Ahmed, et al stated: Exogenous application of SA (0.01 mM), increased dry weight both in saline and non-saline conditions. Under salinity stress, the amounts of Na, Cl and Na/K ratio severely increased and the amount of K, Ca and Mg decreased. Addition of SA in the culture medium inhibited Na^+ and Cl^- accumulation, but stimulated K^+, Ca^{2+} and Mg^{2+} contents of stressed plants. Ben Ahmed, et al suggested SA could be used as a potential growth regulator to improve plant resistance to salinity stress. In studies which have been conducted on Salinity resistance, further has been evaluated the effect of Salicylic acid and less on other organic acid. Therefore, in this study we evaluate effect of Malic acid as an organic acid in creating salinity tolerance also other parameters related to growth.

The aim of this study was to determine the effects of different priming treatments on tolerance of salt stress.

2.0 Material and methods

Plant preparation

The investigation was conducted in May 2011. Seed of faba bean *(Vicia faba L.)* were obtained from Iran (Ilam: Elevation 1339 m, Latitude East 33.638, and Longitude North 46.431). The seeds were sterilized by using 30% hypochlorite for 5 minutes and then washed 3 times with distilled water. For priming, seeds were soaked in aerated solutions of salicylic acid (0.5 and 1 mM) and malic acid (1 and 2 mM) for 24 h. After soaking period the seeds were air dried. primed seeds were sown in plastic pots filled with soil composed of clay and sand (6 seeds in each pot), The plastic pots were divided into two sub-groups; the pots of the first subgroup were irrigated with normal water only to serve as control, while the pots of second sub-group were irrigated with 150 mM NaCl. Day and night lengths were 19/20 h and relative humidity was maintained at 65%. The plants were uprooted 20 days after planting and split up into the root and shoot system. They were rinsed with deionized water and blotted on paper towels before being weighed (fresh weight) [20].

Treatment

Experimental treatment consisted of

1. Control	6. NaCl (150mM)
2. SA (0.5 Mm)	7. SA (0.5 Mm) +NaCl (150mM)
3. SA (1 mM)	8. SA (1 Mm) +NaCl (150mM)
4. MA (1mM)	9. MA (1 Mm) +NaCl (150mM)
5. MA (2mM)	10. MA (2 Mm) +NaCl (150mM)

Measurement

Dry weight (DW) and Na^+/K^+ ratio of root and shoot (g $plant^{-1}$), percentage water content (WC%), leaf area (cm^2), chlorophyll a+b and percentage relative water content (RWC%), MDA content and SOD activity were measured.

Total chlorophyll (a+b) content was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan) which is presented by SPAD value. Average of 3 measurements from different leaves was considered. Oxidative damage to lipids was measured based on the method of Heath and Packer[21]. The activity of superoxide dismutase was measured based on the method of Beauchamp and Fridovich[22]. Proline was quantified by using ninhydrin reagent and measured according to Bates et al [23]. Leaf area was measured by Digital Planimeter. To determine dry weight; the freshly harvested roots and shoots were dried in an aerated oven at 80°C until constant weight. The samples were ground into fine powder and stored in sealed glasses at room temperature for the chemical analysis. The RWC of leaves was calculated by the following equation of Schonfeld et al [23]. Na^+ and K^+ were determined by the flame photometric method [24].

Experimental Design and Statistical Analysis

Plots were arranged in a completely randomized design with 4 replications. Analysis of variance was performed on the data collected using the general linear model (GLM) procedure of the JMP 8 software. The mean separation was conducted by tukey analysis in the same software ($p < 0.05$).

3.0 Result and discussion

The results of related to root traits were shown in Table-1. The highest of dry weight (0.067g plant $^{-1}$), water content (92.11 %) of root were related to SA (1 mM) and the ratio of K^+/Na^+ (4.12 mg g-1 DW) was related MA (2 mM) ($p \leq 0.05$). The lowest of these traits of root were observed in NaCl (150 mM). Table-2 shows, the highest of dry weight and the ratio of K^+/Na^+ (0.233 (g $plant^{-1}$) and 12.36 (mg g^{-1} DW) respectively were related to SA (1 mM) and the highest of water content (92.87 %) was related to MA (2 mM).

Treatment	ROOT *(Vicia faba L.)*		
	Dry weight(g plant^{-1})	**Water content (%)**	**K^+/Na^+ (mg g^{-1} DW)**
Control	**0.05 de**	**89.3 d**	**3 abc**
SA (0.5 Mm)	**0.051 de**	**88.0 f**	**3.78 ab**
SA (1 mM)	**0.067 a**	**92.11 a**	**4 a**
MA (1mM)	**0.053 cde**	**90.17 c**	**3.9 ab**
MA (2mM)	**0.063 ab**	**92.08 b**	**4.12 a**
NaCl (150mM)	**0.032 f**	**82.3 j**	**2.11 c**
SA (0.5 Mm) +NaCl (150mM)	**0.035 f**	**85 i**	**2.5 bc**
SA (1 Mm) +NaCl (150mM)	**0.056 bcd**	**87.3 g**	**3.01 abc**
MA (1 Mm) +NaCl (150mM)	**0.047 e**	**86.17 h**	**2.76 abc**
MA (2 Mm) +NaCl (150mM)	**0.057 bc**	**88.1 e**	**2.95 abc**
Significantly	**	**	**

Table-1: Mean comparisons of Dry weight, water content and K^+/Na^+ ratio of root in response to MA, NaCl and SA and their interaction
Means in each column followed by similar letters are not significantly different at 5% level

The lowest of traits of shoot were related to NaCl (150 mM) ($p \leq 0.05$). According to Table-3, activity of superoxide dismutase enzyme, relative water content and leaf area, with application SA (1 mM) are the higher than (73.21(U g^{-1}Protein), 85.46 %, 24 cm^2 respectively) other treatments also this treatment have the lowest of MDA (µmol/mg protein) ($p \leq 0.05$). The highest of chlorophyll (a + b) was related to MA (2mM) ($p \leq 0.05$). The highest of proline (32.45 µmol.g^{-1} FW) was observed in SA (1 Mm) +NaCl (150mM) also this treatment has the lowest of leaf area (15 cm^2) ($p \leq 0.05$). The lowest of chlorophyll (a + b) and relative water content (1.02 and 66.8% respectively) were observed in Nacl (150 mM) also this treatment have the highest of MDA (102.36 µmol/mg protein) .The lowest of proline (18.16 µmol.g–1 FW) and activity of superoxide dismutase (50.14 U g^{-1}Protein) were related to control.

With increasing SA or MA, traits of root and shoot increased (Table-1 and Table-2). Certainly, the reason of decreasing in root traits is related to decreasing in osmotic potential and accordingly loss of water. As the Table- 1 and Table- 2, application of organic acid are caused resistance to salinity.

Treatment	Dry weight(g plant^{-1})	Water content (%)	K^+/Na^+ (mg g^{-1} DW)
Control	**0.196 a**	**89.12 e**	**8.14 cd**
SA (0.5 Mm)	**0.211a**	**91 c**	**10.6 abc**
SA (1 mM)	**0.233a**	**92.13 b**	**12.36 a**
MA (1mM)	**0.215 a**	**90.12 d**	**10 bc**
MA (2mM)	**0.22 a**	**92.87 a**	**11.9 ab**
NaCl (150mM)	**0.167 a**	**85.8 j**	**5.36 de**
SA (0.5 Mm) +NaCl (150mM)	**0.171 a**	**87.45 i**	**6.04 e**
SA (1 Mm) +NaCl (150mM)	**0.183 a**	**88.36 g**	**6.72 de**
MA (1 Mm) +NaCl (150mM)	**0.17 a**	**87.56 h**	**6 e**
MA (2 Mm) +NaCl (150mM)	**0.178 a**	**88.54 f**	**6.87 e**
Significantly	**ns**	**	**

Ns- Not Significant
Table- 2: Mean comparisons of Dry weight, water content and K^+/Na^+ ratio of shoot in response to MA, NaCl and SA and their interaction
Means in each column followed by similar letters are not significantly different at 5% level

At the cellular level osmotic stress causes alterations in membrane Lipid composition and properties. It has been postulated that at least part of the induced leakiness of membrane is caused by lipid peroxidation resulting from uncontrolled ROS increase (Rodriquez-Rosales, et al., 1999). Measurement of thiobarbituricacid reacting substances (TBARs) concentration such as malondialdehyde (MDA) is routinely used as an index of lipid peroxidation under stress conditions [25]. According to Table-3, the highest of MDA was observed in Nacl (150 mM) that it is in agreement with literature reports. With using of organic acids adjusted salinity effect which is similar to studies of other authors, they stated SA has ameliorative effects in inducing resistance to salinity [26]. Salinity stress affects photosynthetic components such as chlorophylls and carotenoids. Changes in these parameters depend on the severity and duration of stress [27]. Shekari et al [28] reported Seed priming with 2700 µM salicylic acid caused higher relative water content, which protects plants against water stress. In addition to increasing chlorophyll content and stomatal conductance, photosynthetic rates of plant increased. In this investigation, was observed with using of NaCl (150 mM), chlorophyll as the most important pigment of photosynthetic, reduced. The ratio of K^+/Na^+, in salt stress decreased whereas with addition of SA this ratio increased. Ben Ahmed et al reported

Addition of SA in the culture medium inhibited Na^{+} and Cl^{-} accumulation, but stimulated K^{+}, Ca^{2+} and Mg^{2+} contents of stressed plants also. Ben Ahmed et al reported exogenous application of SA (0.01 mM), increased dry weight both in saline and non-saline conditions which it is similar to this experiment.

4.0 Conclusion

The pot experiment was conducted for evaluating of the effect of seed priming with organic acids in tolerance of salt stress. This experiment confirms the other studies and is it agreement with them. This study suggests utilizing of organic acid such as Salicylic acid and Malic acid in salinity condition.

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Treatment	Leaves *(Vicia faba L.)*					
	SOD (U g-1Protein)	relative water content (%)	MDA (µmol/mg protein)	leaf area(cm2)	Proline (µmol.g–1 FW)	Chl a+b (SPAD reading)
Control	50.14 j	75.15 e	92.15 c	19.13 bc	18.16 j	2 b
SA (0.5 Mm)	55.19 i	79.65 d	78.12f	22.14 ab	20.1ci	2.36b
SA (1 mM)	73.21a	85.64 a	35.14j	24 a	24.36h	4.12 a
MA (1mM)	61.15 h	80.54 c	50.14h	20.11b	26g	5 a
MA (2mM)	68.8 d	84.3 b	40.13i	23 ab	26.27f	5.23 a
NaCl (150mM)	62.17 g	66.8 j	102.36a	15.4 cd	28e	1.02 c
SA (0.5 Mm) +NaCl (150mM)	62.8 f	70.12 g	90.15d	15 d	30.1b	1.35 bc
SA (1 Mm) +NaCl (150mM)	70.05b	73.5f	76.5g	15.7 cd	32.45a	1.5 bc
MA (1 Mm) +NaCl (150mM)	66.47e	68.78i	92.3b	15.6 d	28.05 d	1.67 bc
MA (2 Mm) +NaCl (150mM)	69.87 c	70h	80.68 e	16 cd	29.54c	2.07 bc
Significantly	**	**	**	**	**	**

Table -3. Mean comparisons of SOD, relative water content, MDA , leaf area, Proline and Chl a+b of Leaves in response to MA, NaCl and SA and their interaction
Means in each column followed by similar letters are not significantly different at 5% level