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Research Article

De-toxification of sodium in seed germination of Mung bean, *Vigna radiata* by application of Salicylic acid, Ascorbic acid, and Zinc sulfate

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Abstract

Detoxification of Na^+ in terms of germination percentage of mung bean seeds by successive application of salicylic acid, ascorbic acid or zinc sulfate (pre-, post-, or simultaneous application) against the toxic level of NaCl has been carried out in this work. The results revealed that the best case represented by a maximum % of germination when $ZnSO_4$ was supplied protectively i.e. when seeds were pre-treated with $ZnSO_4$ before its exposure to salt stress (NaCl). The germination percentage was 89.3% compared to 84% (as therapeutic way) and 78% (as simultaneous application) of $ZnSO_4$, respectively. Subsequently, the protective approach confirms the same goal (highest germination %) with other substances such as ASA (88%) and SA (86.6%). By contrast with the therapeutic approach, $ZnSO_4$ develops a high response in terms of germination (84%) than ASA (80%) and SA (82.6%). As a conclusion, Zn-salt (Zn as $ZnSO_4$) has a recognizable role in Na-detoxification through different mechanisms on the morphological, physiological, and biochemical levels.

Keywords: Seedlings, Citrus, Foliar spraying, Amino acids, Vegetative growth.

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Introduction

Salinity is one of the problems in crop production. The problem is bigger in Iraq because its soil in the central and southern regions are medium to severe saline. The most dissolved salts in Iraqi soil are sodium chloride, sodium sulfate, magnesium chloride, hydrous-magnesium sulfate, and calcium chloride. The alternative ways need to reclaim saline lands because of its high costs; hence it is necessary to study the physiological and morphological effects of salty land on the different stages of plant growth and production, differences between the various plants in terms of their resistance and sensitivity to salinity, methods and mechanisms of salinity resistance in plants, plant strains resistant to salts and improvement of them by employing genetic engineering. In addition, maintaining the balance of mineral nutrients in plants plays an important role in increasing production and tolerance to environmental stress (Hamdia & Shaddad 2010). Salinity causes two kinds of stress on the plant; the first is water-deficient and the second one is ionic stress that is produced due to a change of Na^+/K^+ ratio.

Mung bean (Vigna radiata *L.*) is sensitive to salinity (Hasanuzzman et al., 2012). Therefore, its exposure to salinity causes many morphological, physiological, and biochemical harmful effects. However, salinity causes oxidative stress by increasing the production of Reactive Oxygen Species (ROS). ROS is toxic to plant cell's causing harmful effects to proteins, lipids, DNA, and the biodefense system that protects this cell and tissue

from salinity (Novo & Parola 2008).

Plants are affected by an increase of sodium ions more than water deficiency due to the negative effect of this element on potassium tendency, enzymes activity, photosynthesis, and bio-metabolism. Sodium is the main cause of harmful effects (Hamdia & Shaddad 2010). It is one of the major challenges facing crop production at a toxic level when growth reduction equals 50%. Hence, this study aimed to alleviate the toxicity of NaCl using organic substances (e.g. salicylic acid (SA) and ascorbic acid (ASA)) and inorganic materials ($ZnSO_4$) and to investigate their physiological effects in seed germination of Mung beans. The findings can help understand the mechanisms of the sensitivity of its seeds to NaCl.

Materials and Methods

Seeds of Mung bean in this study were obtained from Zer Company, Turkey. For this study, five experiments were performed to identify the optimum concentrations of three substances, i.e. salicylic acid, ascorbic acid, and $ZnSO_4$, to alleviate sodium toxicity and the toxic concentration of NaCl, which reduces the germination rate of seeds to approx. 50%. However, the last experiment was designed to understand the effectiveness of these substances in removing sodium toxicity by different methods viz. pre-, post-, and simultaneous as follows: (i) therapeutic method by treating the seeds for 4h with NaCl solution and then adding for another 4h of detoxifying solutions (e.g.: SA, ASA, or $ZnSO_4$), (ii) protective method by treating seeds for 4h with one of the detoxifying solutions (e.g.: SA, ASA, or $ZnSO_4$) and then adding NaCl solution for another 4h and (iii) simultaneous method by adding the NaCl solution and detoxifying solution at the same time, by multiplying the concentration and reducing the volume into half of the substances before mixing for 8h. However, seeds of all treatments were transferred to d/H_2O for an additional 16h. Generally, all methods were done in the following steps successively: (a) washing the seeds with current water for 4h, (b) covering by filter paper (wattman no.1) on each petri dish (3 dishes for each concentration per treatment), (c) using identical 25 seeds for each petri dish, (d) adding 15ml of each concentration in addition to D/H_2O (as control) (SA has been dissolved first in 2ml of ethanol (30%) to prepare the stock solution), (e) the petri-dishes were incubated at 25±1ºC, and (f) the results were recorded by counting the germinated seeds in each dish after 24 h for all concentrations before (Spiegel 1975).

Results

Table 1 shows the germination percentage of the mung bean seed as 82.6-85.3 in distilled water for 24h, while seeds were incubated in SA or AsA solution at concentrations of 10^{-5} and 10^{-6}M had the highest percentage (92 and 93.3%, respectively).

The concentration s of 10^{-5} and 10^{-6}M were considered optimum concentration s of SA or AsA for the following experiments to remove NaCl toxicity. The germination of Mung bean seeds treated with D/H_2O for 24h were 85.3 and 8.8% (different experiments), while the highest percentage was observed (92%) at concentration of 250µg.ml^{-1} for $ZnSO_4$. Therefore, the concentration of 250µg ml^{-1} was considered as optimum concentration of $ZnSO_4$ for the following experiments (Table 2).

The toxic concentration of NaCl was determined by exposing the seeds to a wide range of NaCl conc.s. The results showed that the lowest % of germination was 49.3% at conc. 250µg/ml and this concentration approximately represent the % reduction of germination rate into approx. 50%. Therefore, it was considered the toxic concentration of NaCl in terms of reducing the germination percentage of mung bean seeds.

Table 3 shows the possibility of alleviating the toxicity of sodium using organic compounds having a hormonal nature i.e. SA or as non-enzymatic anti-oxidant (ASA) and an inorganic compound containing Zinc i.e. $ZnSO_4$, in seed germination of Mung bean. Each of these chemicals was processed

Table 1. Determination of optimum concentration of salicylic acid, ascorbic acid on germination percentage of mung bean seeds.

Concentration(M)	Salicylic acid	Ascorbic acid
0.0 (D/H_2O)	82.6	85.3
10^{-10}	86.6	86.6
10^{-9}	82.6	86.6
10^{-8}	85.3	86.6
10^{-7}	84	88
10^{-6}	85	93.3*
10^{-5}	92 *	85
10^{-4}	77	88
10^{-3}	82	84
L.S.D. 0.05	1.31	1.35

Table 2. Determination of optimum concentration ratio of Zinc sulfate & sodium chloride on germination percentage of mung bean seeds.

	% Germination	
Concentration µg ml^{-1}	$ZnSO_4$	NaCl
0.0 (D/H_2O)	85.3	88
5	84	56
10	85	57
25	84	54
50	85.3	53
100	78	58
250	92 *	49.3 *
500	88	61
1000	93	60
2000	82.6	56
L.S.D. 0.05	1.35	2.88

Table 3. Detoxification of NaCl in terms of germination % of mung bean seeds by successive application of SA, ASA, and ZnSO4 as protective, therapeutic, and simultaneous applications.

Treatments	Germination %
8h, D/H_2 (general control)	88
8h, NaCl, 250 µg ml^{-1} (toxic)	54.6
8h, SA ,10^{-5}M (SA control)	84
8h, ASA, 10^{-6}M, (ASA control)	89.3
8h, $ZnSO_4$,250 µg ml^{-1},($ZnSO_4$control)	88
4h,SA→4h,NaCl (protective way)	86.6
4h, NaCl → 4h,S (Therapeutic way)	82.6
8h, SA + NaCl (simultaneous way)	81.3
4h,ASA → 4h,NaCl (protective way)	88
4h, NaCl → 4h, ASA (Therapeutic way)	80
8h, ASA + NaCl (Simultaneous way)	81.3
4h, $ZnSO_4$ → 4h, NaCl (protective way)	89.3
4h , NaCl → 4h, $ZnSO_4$ (Therapeutic way)	84
8h, $ZnSO_4$ + NaCl (Simultaneous way)	78.6
L.S.D = 2.35	

in optimum concentrations to the seeds in three ways: (a) protective way (pre-treatment) for 4h.t, (b) therapeutic way (post-treatment) for four hours, and (c) simultaneously with toxic sodium, applied together at the same time for 8h.

The results showed the highest percentage of germination (89.3%) was obtained by applying $ZnSO_4$ in a protective way i.e. the application of zinc sulfate to seeds before exposure to salt stress is the toxic level of NaCl (Table 3). The seeds revealed a germination percentage of 89.3% (in protective way) compared to 84% (in therapeutic way) and 78% when

applied simultaneously. However, the protective method it gives the same results (highest values) with SA and ASA but to in the lower levels (86 and 88%, respectively). In addition, the comparison of the effectiveness of the used substances when supplied therapeutically: means treating the seeds with a toxic concentration of NaCl for 4h then adding any of the other substances for another 4h has given the highest germination percentage with $ZnSO_4$ 84% compared to 80% for ASA and 82% for SA. The Zn salts as $ZnSO_4$ have an important role in detoxifying Na in the first place through a protective way.

Discussion

The toxic level of NaCl was determined in mung bean seeds based on the reduction of growth rate in terms of germination percentage into approx. 50%. The germination % was reduced to 49.3% at 250µg ml^{-1} of NaCl compared to control treatment (D/ H_2O = 88%). This agrees with Hasegawa et al. (2000) that showed Na+ is toxic and harmful in most plants when its level is exceeded 1-10mM. However, morphological and physiological evidence reduce of growth rate by 50%, such as chlorophyll content (Jaleel et al. 2008), leaf area (Munns 2002), cell expansion (Hu et al. 2005), IAA level (Hopkins & Hüner 2009; Shaheed & Merhij 2016), and rate of transpiration (Shaheed & Merhij 2016). The increased Na^+ content severely reduced crop production, and the harmful effects of high Na^+ concentration in the medium of plant can be interpreted in three different ways: (1) inhibition of water uptake due to osmotic potential of the culture solution (lea-cox & Syvertsen 1993), (2) disturbance of normal metabolism caused by high concentration in plant tissue (Crammer et al. 1991), and (3) inhibition of the absorption of other essential cations by plants (Cachorro et al. 1994).

Salinity creates an appropriate circumstance for entering the toxic ions into the seed embryo, delaying the germination time, lowering the seedling growth rate, and reducing the germination percentage (Aceves et al. 1975). The first germination step is the imbibition of water, which causes natural, chemical, and biological changes successively, promoting enzymatic activity and cell division. However, inhibition of water uptake, increasing osmotic pressure of external solution by salinity inhibit the whole process of germination (Hunter & Ericksion 1952). For example, at 200mM NaCl, the germination of soybean T49 is prevented completely (Mukhiya et al. 1981). Thus, the failure of the seed to germinate is attributed to the osmotic pressure of the germination medium more than for the cell itself (Novikov 1942). Increasing Na content in plants exposed to NaCl, cause ionic imbalance and metabolic toxicity by competition between Na^+ and K^+ on binding sites of many enzymes (Tester & Davenpert 2003). Toxic Na^+ reduces K^+/Na^+ ratio in safflower under salt stress (Hosseini et al. 2010). Many factors cause ions loss, such as lipid peroxidation, mechanical defects, and K^+ efflux channels. The latter should be the dominant mechanism (Demidchik 2010).

Salinity is abiotic-stress causing oxidative stress, which regenerates ROS as a secondary product of metabolism. The plasma membrane is the primary site of salt injury (Mansour 1997). Thus, Na^+ toxicity mainly affects the cytoplasm membrane by inducing lipid peroxidation and protein destruction by activating lipoxygenase and protease enzyme, respectively (Merhij 2016), thereby causing permeability perturbation. In addition, Na^+ toxicity causes a decline of IAA concentration by increasing IAA-oxidase at the same time in cucumber (Merhij 2016). Notwithstanding, salicylic acid and ascorbic acid decrease lipids peroxidation by affecting the number of productive H_2O_2 (Ganesam & Thomson 2001).

The role of SA, AsA, and Zn in alleviating Na-toxicity has been studied through an interaction between the optimal concentration of these substances (10^{-5}M, 10^{-6}M, and 250 µg/ml), and the toxic level of NaCl (250µg/ml), in terms of germination percentage of mung bean seeds. However, all these substances have protective effects

in developing anti-stress programs to accelerate natural growth after removing stress factors (Sakhabutdinova et al. 2003). Detoxification of NaCl results was obtained by successive application of SA, AsA, and $ZnSO_4$ in three different ways pre-, post-, or simultaneous application with the toxic NaCl. The results showed the possibility of detoxifying Na^+ using organic compounds with hormonal nature (salicylic acid) or non-enzymatic antioxidants (ascorbic acid), and an inorganic micronutrient, Zn ($ZnSO_4$), in seed germination. The results also revealed the best developmental case represented by a maximum % of germination when $ZnSO_4$ is applied protectively i.e. when seeds were pre-treated with $ZnSO_4$ (priming) before their exposure to salt stress (NaCl). The germination percentage was 89.3% compared to 84% (therapeutic way) and 78% (simultaneous application) of $ZnSO_4$, respectively. This germination % (89.3) is highly significant compared to the toxic level of NaCl (54%). Meanwhile, it does not differ from the germination % of seeds treated with distilled water (88%) i.e. the protective method for applying $ZnSO_4$ completely prevents all the processes that deal with the effects of toxic Na. Moreover, by contrast with the therapeutic approach, again, $ZnSO_4$ develops a higher response in terms of germination percentage (84%) while ASA (80%)and SA (82.6%).

SA, AsA, and $ZnSO_4$ have a recognizable role in Na^+ detoxification through different mechanisms on the physiological and biochemical levels. It is well-known the role of ascorbic acid in AsA–GSA cycle of oxidative stress (Foyer & Halliwewl 1976). AsA is an antioxidant acting as a scavenger for ROS directly (Thomass 1992) and for H_2O_2 indirectly via the activity of AsA-peroxidase (APX) (Asada 1992). However, AsA plays an important role in regenerating α-tocopherol (vitamin E) for maintaining cellular membrane (Noctor & Foyer 1998).

Many studies reported the protective role of SA in the germination process of *Triticum aestivum* (Shakirova et al. 2003). Lee et al. (2005) pointed out the role of SA in the reduction of an inhibitory effect of salinity on germination as well as under osmotic stress (Borasani et al. 2001) through its influence on ROS particularly H_2O_2. SA, increased the uptake of K^+ and decreased the uptake of Na^+ under salinity stress, ultimately maintaining the cellular membrane integrity of tomatoes (Abdi et al. 2011).

Zinc is an essential micro-nutrient for the growth and development of crops and acts on the reduction of uptake of some elements (Salardini et al. 1992). In addition, Zn is considered as part of many enzymes such SOD, CAT which acts as an enzymatic antioxidant in the defense system (Marschner 1988). However, $ZnSo_4$ has the dominant effect compared to other Zn-salts such as chloride and nitrate in the percentage of seed germination (unpublished data). Zn deficiency caused interruption of auxin metabolism because of its role in tryptophan biosynthesis, which acts as a precursor for IAA (Blazich 1988). Szydl & Pachokzak (2007) pointed out the role of Zn in the induction of rooting in stems of ornamental trees, as a common practice primarily dependent on IAA.

It is well-known that Zn ion acts as a strong inhibitor for NADPH- oxidase in root cells of Phaseolus and Cotton. This indicates that salt (excess of Na^+) induced regeneration of superoxide (O^-_2) by NADPH-oxidase, which was strongly inhibited by Zn. For controlling Na-toxicity depending on the application of $ZnSo_4$ before supplying the toxic–Na has a complete protective role in Na-detoxification developing a percentage of seed germination equal to that of seeds treated with d/H_2O (un-stressful seeds). Finally, as a conclusion raised from the current study, it is possible to use Zn in regulation and improving plant growth and development in stressful plants. The role of Zn in particular and then SA and ASA reside in hormonal balance and its reflections on membrane repairing in the first place and control permeability perturbation via its oxidative damage of phospholipid, protein, and DNA through its effects in many physiological and biochemical processes in term of Na^+-detoxification. Finally, few biological

processes occur earlier during seed germination. Such processes might be inhibited partially by sodium and possibly detoxified by $ZnSO_4$ early during the first 4h or later by SA or ASA applied successively during the second 4h, which might be the aims of the next manuscript.

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