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Research Article

Response of Salicylic Acid on Seed Germination and Physio-Biochemical Changes of Wheat Under Salt Stress

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Abstract

Due to natural processes and anthropogenic, the soil is rapidly contaminated with the continuous accumulation of salts, which affects the crop production worldwide. Therefore, it is essential to exploit the modern techniques to improve the tolerant of plants to salinity. The present experiment was designed to study the effect of SA on (1) seed germination parameters and (2) physio-biochemical changes in wheat seedlings under NaCl stress. NaCl exhibited reduced germination parameters (germination %, vigor index and mean germination time), seedlings height, content total chlorophyll and total carbohydrates and increased production of reactive oxygen species [detected using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) for H_2O_2 and dihydroethidium (DHE) for O_2•-] in roots, content of malondialdehyde and proline, and chlorophyll degradation. However, wheat seedlings treated with SA showed increased seed germination traits and plant height by increasing proline and total soluble carbohydrates by suppressing ROS formation in roots and leaf-chlorophyll degradation.

Keywords: Wheat; Proline; Total Soluble Carbohydrates; Reactive Oxygen Species; Chlorophyll Degradation

Introduction

Salicylic acid (SA) is one of the hormone-like endogenous regulators that is present in plants either in a free state or in the form of methylated glucose-ester, glycosylated, or amino acid conjugates [1]. It acts as a signaling molecule and affects many physiological and biochemical processes under abiotic stress and non-stress conditions. SA influences seed germination, establishment of seedling, cell growth and expansion and also stimulates the activity of enzymes; synthesis of flavonoid and photosynthesis process under adverse environmental conditions [2-5].

It is well established that SA plays several new roles in plants as well as animals. Several investigations proved that SA is involved in tolerance of salt stress [6], water stress [4] and heavy metals [7-9]. SA improves salt stress resistance in soybean [6], rice [10], *Dianthus superbus* L. [11], and in cabbage [12]. However, the processes of physiological and biochemical involved in tolerance of plants to salt stress are enhanced by SA still needs to be studied further [13,14].

Among the abiotic stress, salt stress limits the plant growth and development by disturbing many physiological and biochemical processes, such as osmotic adjustment, nutrient homeostasis, biomolecules synthesis, photosynthesis, respiration, enzymes activity and water balance [15]. In many studies reported that application of NaCl decreased seed germination in eggplant [16] and *Origanum compactum* (Benth) [17]. Salt stress suppresses not only seed germination but also affects root and shoot growth and root and shoot fresh weight of four vegetable species [18]. Therefore, present experiment was aimed to study the effect of SA on (1) seed germination parameters and (2) physio-biochemical changes in wheat seedlings under NaCl stress.

Materials and Methods

Preparation of seeds and treatments

Under laboratory conditions, an experiment was performed on wheat (*Triticum aestivum* L. var. 'Samma') obtained from a local market of Riyadh, Saudi Arabia. The sterilized seeds were placed in Petri dish (Size 12 in) having a double layer of filter papers. One hundred fifty sterilized seeds were placed in each Petri Dish and all Petri dishes were arranged in a simple randomized design with single factor and 4 replicates. Treatments of SA were applied with and without NaCl as follows (1) 0 µM SA + 0 mM NaCl (control), (2) 100 µM SA + 0 mM NaCl, (3) 0 µM SA + 100 mM NaCl and (4) 100 µM SA + 100 mM NaCl. After supplying treatments, each Petri Dish was sealed with paraffin tape to avoid evaporation. The Petri dishes were kept inside an incubator at 28 ± 3°C.

Germination of seeds was noted every day. After every 3 d, all treated seedlings were transferred on sterile filter papers in Petri dish. Same concentrations and volume of treatments were supplied. The potential of seed germination was assessed in terms of percent seed germination, mean germination time and vigor index (VI). The physiological and biochemical characteristics of seedlings of wheat were measured.

Determination of growth characteristics

The seed germination rate was recorded every day from 2 to 14 d. Seeds were considered as germinated when their radicle showed at least 2-mm length.

Germination percentage (GR%) was calculated with the suitable formula:

Germination (%) = (Number of seeds sprouted/Total number of seeds) ×100

Mean germination time and seedling vigor index were determined according to the method described by Matthews and Khajeh-Hosseini [19] and Vashisth and Nagarajan [20], respectively.

At the end of the 14 d, samples were taken for physiological and biochemical parameters determination.

Histochemical detection of ROS in roots of wheat seedlings

A fluorescent probe 2'-7'- dichlorofluorescein diacetate (DCF-DA) was used to detect H_2O_2 in root of wheat seedlings according to the method described by Tarpey., *et al* [21]. Roots image was taken using a fluorescence microscope at excitation and emission wavelengths of 480 and 530 nm.

The dihydroethidium (DHE) was used to detect superoxide radicals (O_2•-) in the roots wheat seedlings following the method described by Rodriguez-Serrano., *et al* [22]. The signal of DHE was captured in the roots as red fluorescence (490 nm excitation; 520 nm emission).

Total soluble carbohydrates content

Total soluble carbohydrates (TSC) concentration was estimated by taking absorbance at 490 nm, as described by Dubois., *et al.* [23], using glucose as a standard. TSC was presented as mg g^{-1} dry weight (DW).

Malondialdehyde content

To measure lipid peroxidation in seedlings, malondialdehyde (MDA) content was determined using the procedure of Dhindsa., *et al* [24]. MDA was calculated according to Heath and Packer [25].

Proline content

The content of proline in the leaf tissues of wheat seedlings was measured spectrophotometrically following the method described by Bates., *et al.* [26]) via reaction with ninhydrin.

Protein isolation and SDS-Page

Leaf of wheat seedlings was taken and powdered in liquid nitrogen using mortar and pestle. Protein was isolated by homogenizing the leaf powder in a 1.0 mL lysis buffer containing 25 mM HEPES buffer (pH 7.5), 500 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 0.2% Nonidet P-40 (v/v) and 1 mM PMSF. Cell debris was pelleted by centrifugation at 12,000 rpm for 10 minutes at 4°C. Protein was quantified according the method described by Bradford [27], protein content was measured with BSA as a standard. An equal amount of each protein sample was resolved on 7.5% SDS-PAGE and electrophoresed at 70 V using a Bio-Rad Mini-Protein Tetra Cell, 4 Gel System (Bio-Rad Laboratories, Hercules, USA).

Statistical analysis

All the treatments had four replicates and each Petri dish was treated as one replicate. The statistical analysis was performed using SPSS v17 statistical software (SPSS Inc., Chicago, IL, USA). The data were expressed as means ± standard error and means were statistically compared by Duncan's multiple-range test (DMRT) at the $p < 0.05\%$ level.

Results

Under both conditions (salinity and non-salinity), application of SA proved best for all germination parameters (Figure 1A-C). Treatment of SA increased percent germination by 9.07%, vigor index by 26.01% and mean germination times by 31.55 over the respective controls under non-stress condition. However, application of NaCl decreased all germination traits as compared to control. While, application of SA increased percent germination by 13.05%, vigor index by 68.60% and mean germination times by 39.74% over the respective NaCl treatments. Figure 2 exhibits that application of SA improved plant length under both salinity and non-saline conditions.

Figure 1: Effect of salicylic acid on (A) percent germination, (B) vigor index and (C) mean germination time of wheat.

Figure 2: Effect of salicylic acid on the length of wheat seedlings under NaCl stress.

Figure 3 shows that production of H_2O_2 and O_2•- was detected in root of SA treated-wheat seedlings under salt stress and non-stress conditions. Under non-stress condition, application of SA exhibited low levels of green and red signals for H_2O_2 and O_2•- respectively as compared to control and NaCl treatment. The highest levels of signal were detected under NaCl stress condition. However, application of SA exhibited a weak signal as compared to NaCl treatment.

Figure 4

Figure 3

Figure 3: In situ visualization of ROS formation in primary roots using the fluorescent probe DCF-DA and DBE for (A) H_20_2 and (B) (0_2•-) respectively under salinity stress.

Figure 4: Effect of salicylic acid on (A) Total Chlorophyll and (B) chlorophyll degradation of wheat.

Application of SA significantly decreased accumulation of malondialdehyde in wheat seedlings under both salt stress and non-stress conditions (Figure 4). However, application NaCl substantially increased malondialdehyde content. While, application of SA decreased malondialdehyde content by 39.31% over the NaCl treatment.

Application of SA proved best for the biosynthesis of Total chlorophyll under both conditions (Figure 5A). Application of SA increased Total chlorophyll by 35.43% over the control. However, application of NaCl decreased Total chlorophyll content. While, application of SA increased 74.05% over the NaCl treatment. Figure 5B shows that the lowest degradation of chlorophyll was recorded in wheat seedlings treated with SA under both salt stress and non-stress conditions. Application of SA decreased chlorophyll degradation by 43.19 under non-stress, and by 67.37% under salt stress condition.

Figure 5: Effect of salicylic acid on (A) Total Chlorophyll and (B) chlorophyll degradation of wheat.

Figure 6 (A-B) reveals that application of SA significantly increased proline and Total soluble carbohydrates accumulation as compared to the control. Also, application of NaCl increased proline accumulation. However, under NaCl stress, application of SA improved further accumulation of proline. Under non-stress, application of SA increased proline by 65.31% over the control. While, under NaCl stress, application of SA increased proline further by 116.21% over NaCl treatment. Application of SA increased Total soluble carbohydrates content by 66.68% over the control under non-stress condition, while NaCl treatment increased Total carbohydrates content as compared to control. Moreover, application of SA increased Total soluble carbohydrates content by 25.86% under NaCl stress.

Figure 6: Effect of salicylic acid on (A) proline content and (B) Total soluble carbohydrates content in leaf of wheat.

Changes in proteins profile in the leaves of wheat plant seedling treated with SA under NaCl stress and non-stress conditions were analyzed by SDS-PAGE as shown in figure 7. Under NaCl stress, treated seedlings showed 4 proteins bands with molecular weight 180, 39, 37 and 15 KDa as compared to control. Under NaCl stress,

wheat seedlings exhibited 2 bands of proteins with low molecular weight i.e. 15 and 12 KDa as compared to NaCl treated plants.

Figure 7: Effect of salicylic acid on protein profile of wheat under NaCl stress. Leaf proteins extracted from different treated plants were ran in separate lanes after loading equally to each lane.

Discussion

Under both salinity and non-salinity conditions, application of SA significantly improved germination, physiological and biochemical characteristics of wheat seedlings (Figure 1-6). However, salinity reduced all these parameters except content of malondialdehyde, proline and chlorophyll degradation.

Healthy seed germination provides a foundation for better plant growth and development under normal and different environmental conditions. In the present study, application of NaCl impaired the seed germination characteristics (Figure 1A-C). It may be due to low water potential that prevent water uptake and nutrients availability for germination [28,29]. Also, high salt in solution may cause toxicity to embryo [28]. The seed germination is also inhibited by reduced α-amylase activity due to salinity via reduced bioactive gibberellin content [30]. Interestingly, application of SA improved all germination characteristics (percent germination, germination vigor and mean germination time) under both conditions. An increase in these traits may due to the roles of SA in increasing of oxygen, nutrients uptake, and activity of α-amylase activity.

In this investigation, we observed that the height of wheat seedlings inhibited with the application of NaCl (Figure 2). It may be due to the inhibitory effects of NaCl on plant growth metabolisms. However, application of SA proved beneficial in improving seedlings height under both salt stress and non-stress conditions. Basal application of SA through the rooting medium exhibited an ameliorating and growth inducing effects under stress and non-stress conditions by decreasing over-production of ROS in roots (Figure 3). An increase in seedlings height may be due to the roles of SA in nutrients mobilization and also an accumulation of abscisic acid and indole-3-acetic acid, resulting in improved protective and promoting effects of SA [15,31].

The accumulation of ROS (H_2O_2 and O_2•-) is the reason for the oxidative damage in plants under stresses. Also, malondialdehyde accumulation represents a marker for lipid peroxidation. The accumulation of ROS in roots and malondialdehyde content in the leaf of wheat seedlings increased under NaCl stress (Figure 3 and 4). Exogenous supply of SA to rooting medium significantly suppressed the formation of ROS and malondialdehyde accumulation. It may be due to the accumulation of proline (Figure 6A) and antioxidant system, as they are responsible for ROS scavenging, resulting in increased tolerance to salinity by reducing lipid peroxidation [15,31].

Biosynthesis of chlorophyll in plant improves photosynthesis results better dry matter production in plants. Under NaCl stress, wheat seedlings showed decreased total chlorophyll content and increased chlorophyll degradation (Figure 5A and B). However, SA supplied to the growth medium improved the accumulation of total chlorophyll and suppressed chlorophyll degradation. It may be due to the protective role of SA that decrease chlorophyll degradation through regulating ascorbate and glutathione pool [33]. An increase in total chlorophyll content provides better photosynthesis, which may be one of the reasons for better seedlings height (Figure 2).

Under NaCl stress, accumulation of proline, total soluble carbohydrates content and proteins was significantly affected (Figure 6A-B and 7). NaCl-treated wheat seedlings exhibited reduced total carbohydrates content and increased proline content. However, seedlings treated with and without NaCl improved total soluble carbohydrates and also proline content. The levels of increase in the content of total soluble carbohydrates and proline in leaves under salinity and non-salinity might be due to SA that increases photosynthesis by increasing chlorophyll (Figure 5A), activity of Rubisco and CA, and decreasing degradation of Chls (Figure 5B) [34]. Proline acts as an antioxidant by scavenging hydroxyl radical and protecting the structure and function of DNA, protein and membranes [35]. According to Azooz., *et al.* [36] that the accumulation of proteins may be used by the plants to fight against the salinity.

Conclusion

It can be concluded on the basis of obtained results that plant treated with NaCl exhibited reduced germination parameters, seedlings height, content of total chlorophyll and increased total carbohydrates, production of ROS (H_2O_2 and O_2•-) in roots, content of malondialdehyde and proline, and chlorophyll degradation. However, wheat seedlings treated with SA showed increased seed germination traits and plant height by increasing proline and total soluble carbohydrates by suppressing ROS formation in roots and leaf-chlorophyll degradation.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Author's Contribution

All the authors contributed equally to this manuscript.

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