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Effect of Salt Stress on Plant Growth and Free Endogenous Hormones of Primed Radish (*Raphanus Sativus* L.) Seeds With Salicylic Acid

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Abstract : Factorial experiment with two factors was conducted to study the effect of radish seeds (*Raphanus sativus* L.) primed with salicylic acid (SA) for 24 h on plant growth and hormones concentration and its ability to increase plant tolerance to salt stress added through water irrigation as sodium chloride (NaCl). The results showed that salt stress was negatively affect on plant height, chlorophyll content, fresh weight of shoot and root and, free IAA concentration. While, salt stress inefficient on GA and CK concentrations. Priming seeds with SA caused a decrease in free IAA, GA concentrations, while free CK and ABA concentration did not different significantly from control plants which gives the plant more tolerance to salt stress.
Key words : salt stress, radish, salicylic acid, endogenous hormones.

Introduction

Radish (*Raphanus sativus* L.) is originally from Europe and Asia. It grows in temperate climates at altitudes between 190 and 1240 m. It is 30–90 cm high and its roots are thick and of various sizes, forms, and colors (1). Cytokinin (6-benzylamino-9-glucosylpurine) is a major metabolite of 6-benzylaminopurine (6-BAP) in the root radish (2). Eleven gibberellins have been identified in extracts of mature seed as 13 hydroxy-GAs and four gibberellins have been identified as non-13-hydroxy-nGAs (3).

Salt treatment significantly decreased shoot and root fresh and dry weight, emergence percent (EP), leaf number per plant (LNPP), leaf relative water content (LRWC), chlorophyll and mineral content, but increased electrolyte leakage (EL) (4). The salinity reduced germination percentage and also delayed the germination rate as salt level increased. Lengths and fresh weights of root and shoot decreased with the increasing of salt concentration. Furthermore, photochemical efficiency of PS_2, photochemical quenching coefficient non photochemical quenching coefficient, leaf area and chlorophyll content (SPAD value) were reduced by salt stress. (5). At salinities higher than 4 ds m^{-1}, total plant dry weight decreased 2.8% per ds m^{-1}. About 80% of the growth reduction at high salinity could be attributed to reduction of leaf area expansion and hence to reduction of light interception. The remaining 20% of the salinity effect on growth was most likely explained by a decrease in stomatal conductance (6). Physiological parameters such as net photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal CO2 conc. and water use efficiency was decreased in both radish varieties under salt stress (7).

SA is an endogenous growth regulators of phenolic nature, which participates in the regulation of physiological processes in plant (8). It significantly increased the fresh and dry weights in root and shoots of wheat plants under salt stress. Similarly, it promoted the activity of antioxidative enzymes (9). Salicylic acid pretreatment alleviated the adverse effects of salinity stress on germination percentage, length of shoot, fresh and dry weight, photosynthetic pigments and K^+ concentration (10). Plants treated with SA showed no recovery from excessive accumulation of Na^+ in their shoot to root ratio, when under salt stress (11).

Plant hormones are comprised of a group of structurally unrelated small molecules that regulate a wide variety of plant processes. The hormones also act to integrate diverse environmental cues with endogenous growth programs. So far, ten phytohormones have been identified including auxin, abscisic acid (ABA), cytokinin (CK), gibberellins (GA), ethylene, brassinosteroids (BR), jasmonate (JA), nitric oxide, and strigolactones (12, 13, 14, 15, 16, 17, 18). Plants also utilize several peptide hormones to regulate various growth responses (19).With the application of biochemical, genetic, and genomic approaches, many aspects of hormone biology have been elucidated, especially in the model flowering plant Arabidopsis thaliana.

Most hormones involve in multiple biological processes and influence on each other through elaborate crosstalk strategies to elucidate hormone-signaling pathways (20).

The objective of the present study was to observe the effect of the priming radish seeds with SA on some physiological parameters and the endogenous hormones under saline and non-saline conditions.

Materials and Methods

Seeds of radish (*Raphanus sativus* L.) were primed with different concentration of SA ($0,10^{-4},10^{-5}$M) for 24 hour and grown in growth cabinet of science department of Babylon University. Germination percentage was taken after 10 days. Then ten days old seedlings were planted in plastic pots containing 5 kg of soil:peatmose 1:1 (three pots for each treatment). At first ten days, seedlings were irrigated with tap water 1.3 dS m^{-1} (control treatment) twice a day; then they were irrigated with salted water (5 and 10 dS m^{-1} NaCl) every day up to 90th day. Plant length, root and leaf fresh weight were measured. Chlorophyll content measured by chlorophyll meter (Monilta, SPAD-502Plus) and leaf area measured by planimeter **(**Placom KP90 Digital) **.**

Free plant hormones were determined according to Ergun et al. One gram of fresh or dry weight of leaf sample was taken and combined with 60 ml of methanol:chloroform:2N ammonium hydroxide (12:5:3 v/v/v). Each combined extract (60 ml) was kept in a bottle at -20°C in deep freeze for further analysis. The combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated. The water phase was adjusted to pH value of 2.5 for IAA , GA3 and ABA extraction and pH value of 7 for zeatin extraction with 1 N HCl and 1 N NaOH, respectively. After that, 15 ml ethyl acetate was added and extracted three times to isolate free-form of IAA, GA3, ABA and zeatin. Spectrophotometric assay was done using 222 nm and 280 nm wave lengths for IAA, 254 nm for GA3, 263 nm for ABA, and 269 nm for zeatin and for all standard synthetic IAA, GA3, ABA and zeatin and isolated samples(21).

The data were analyzed statistically with SPSS-17 statistical software. Means were statistically compared according to Least Significant Difference ($LSD_{0.05}$) (22) LSD test at $p< 0.05$ level.

Results

Figure 1 showed the interaction between salt and SA concentrations. It is clarified that salt concentrations (2.5, 5, 10 dS m^{-1}) caused a significant increase in radish seeds germination percentage about (25, 29, 12.5%) respectively. SA-primed seeds treated with 1.3 and 10 dS m^{-1} NaCl showed a significant decrease in germination percentage. In reverse, SA-primed seeds treated with 2.5 and 5 dS m^{-1} NaCl caused a significant increase in seeds germination percentage.

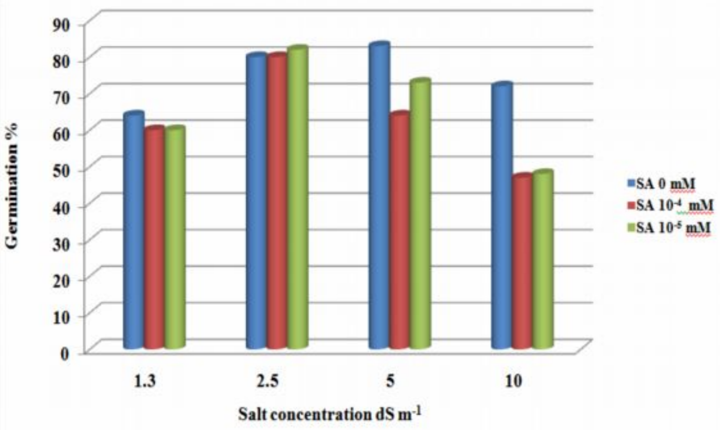

Figure 1:The effect of salt and SA concentration on germination.

LSD 0.05 = 1.7

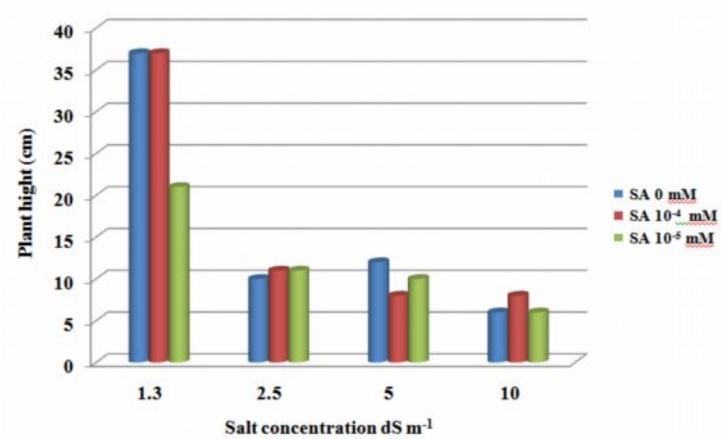

Figure 2: The effect of salt and SA concentration on plant length (cm).
LSD $_{0.05}$ = 4.4

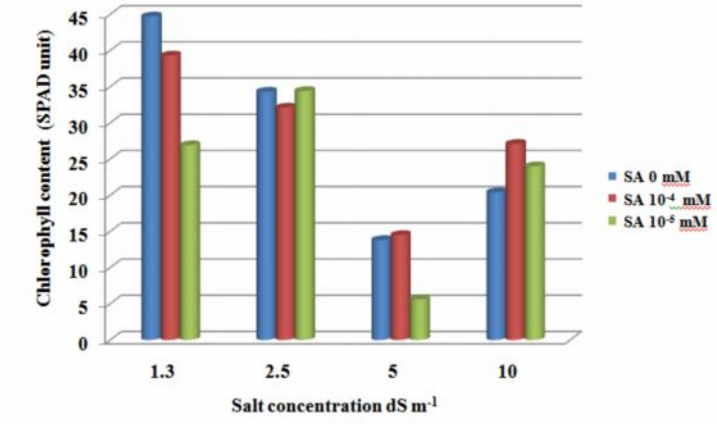

Figure 3: The effect of salt and SA concentration on chlorophyll content.

LSD $_{0.05}$ = 7.5

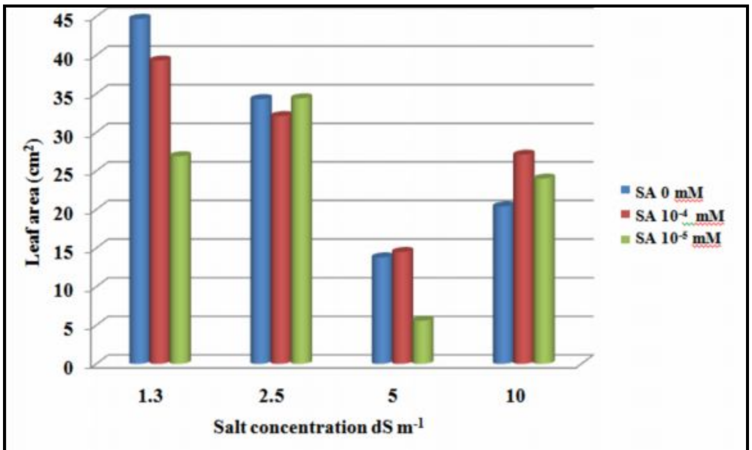

Figure 4: The effect of salt and SA concentration on leaf area.
LSD $_{0.05}$ = 25.9

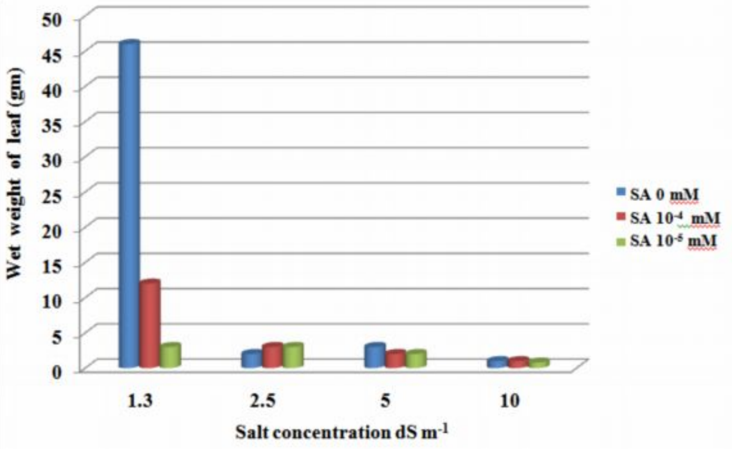

Figure 5: The effect of salt and SA concentration on wet weight of leaves.
LSD $_{0.05}$ = 3.5

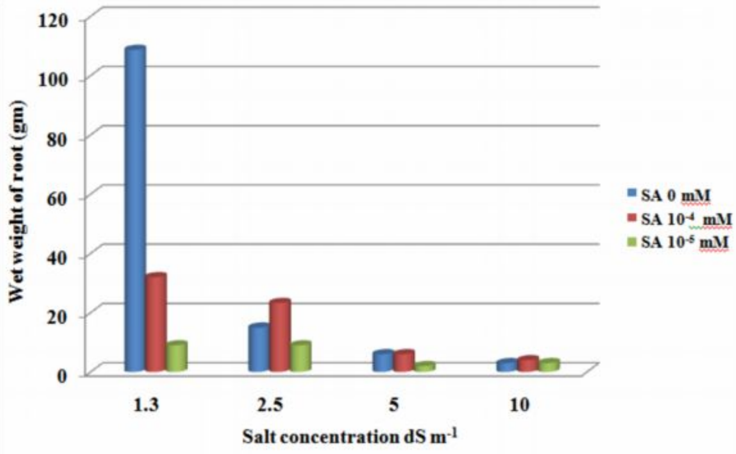

Figure 6: The effect of salt and SA concentration on wet weight of roots.
LSD $_{0.05}$ = 7.8

Salt stress with SA combination showed a significant decrease in physiological parameters of radish plants which include plant length, chlorophyll content, leaf surface area, wet weight of leaves and wet weight of roots (Figures 2-6).

Figure 7 showed the interaction between salt and SA concentrations in endogenous free IAA concentration. It was clarified that salt concentrations caused a significant decrease in IAA concentration both plants grown by primed and unprimed seeds.

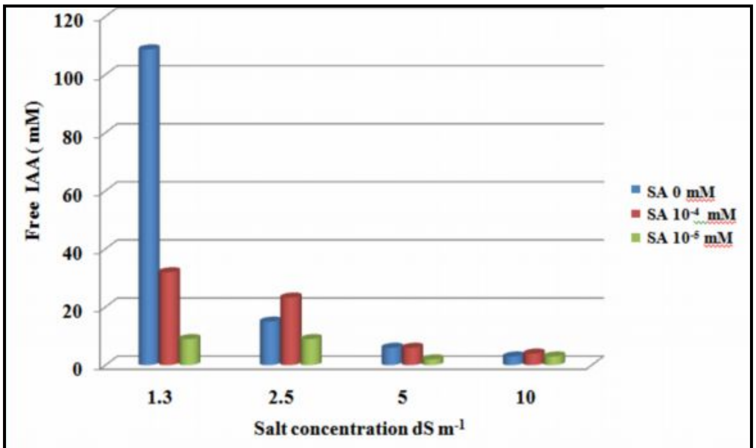

Figure 7: The effect of salt and SA concentration on free IAA.

LSD $_{0.05}$ = 0.05

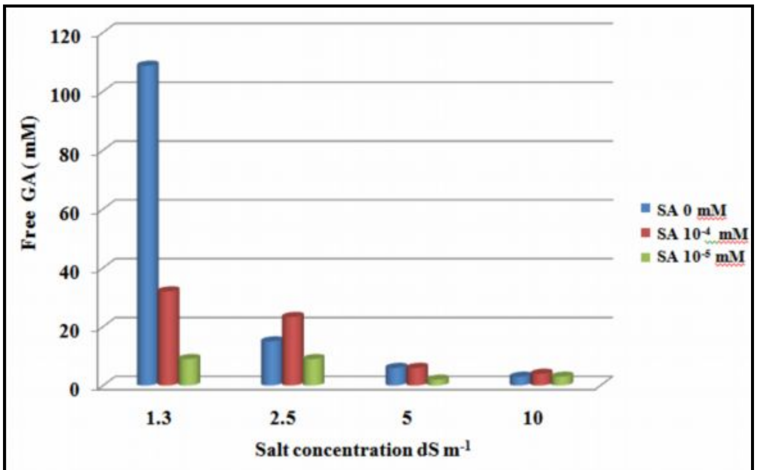

Figure 8: The effect of salt and SA concentration on free GA.
LSD $_{0.05}$ = 1.1

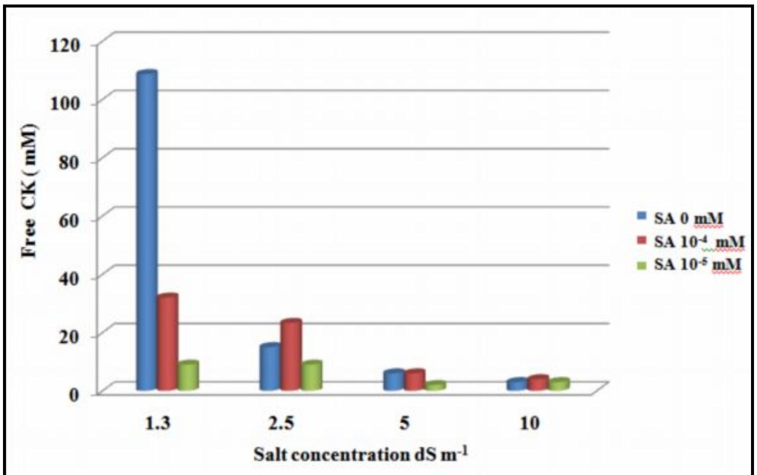

Figure 9: The effect of salt and SA concentration on free CK.
LSD $_{0.05}$ = 0.7

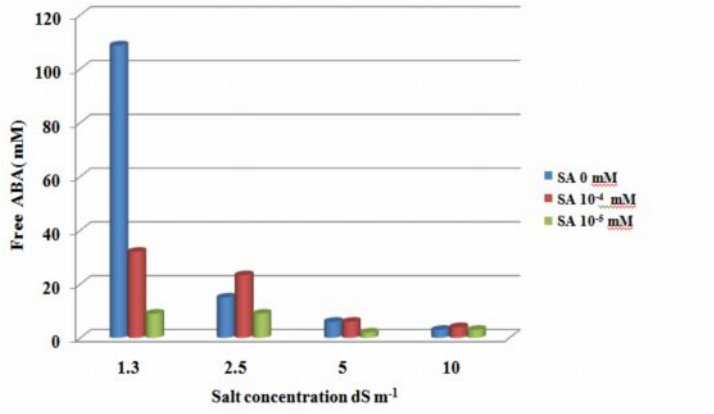

Figure 10: The effect of salt and SA concentration on free ABA.
LSD $_{0.05}$ = 12.6

Figure 8 showed that salt concentrations did not affect GA concentration. Plants grown from 10^{-4} SA-primed seeds showed a gradual decrease in GA concentration which was in agreement with salt concentration increase While plants grown from 10^{-5} SA-primed seeds had no significant changes. CK concentration had no significant effects on both plants grown from primed and unprimed seeds even exposed to salt stress (Figure 9). The same results were found in ABA concentration except for 10^{-4} M SA which caused a significant decrease in ABA concentration in plants stressed by 5 and 10 dS m^{-1} NaCl (Figure 10).

Discussion

In this research, we found that salt stress caused a deleterious morphological and cellular effects. This result was compatible with (23) who concluded that the effects of stress are often manifested at morpho-physiological, biochemical and molecular levels, such as inhibition of growth (Figures 2-6) and changes in endogenous contents of phytohormones (24, 25, 26, 27). It appears that the increase in germination percentage is due to absorb more water in lower water potential environment caused by higher salt concentrations (Figure 1). This result shows ecological implications that might be related to the role of high concentrations of gibberellins in radish seeds which exist after seed germination (Figure 8) (28). This consequence is compatible with (3) who identified eleven kinds of gibberellin in extracts of matured seed as 13 hydroxy-GAs (GA1, 3-epi-GA1, GA8, GA9, GA17, GA19, GA20, GA24 12β-hydroxy-GA24, GA25 and GA77) and four non-13-hydroxy-nGAs (GA9, GA24, 12β-hydroxy-GA24, GA25). While seeds treated with SA showed a significant increase in their germination (Figure 1). It concluded that although SA is not essential for germination under normal growth conditions, it plays a promotive role in seed germination under high salinity, probably by reducing oxidative damage (29).

Plant hormones such as auxin (indole-3-acetic acid), gibberellins, cytokinins, and abscisic acid have central roles in plant growth regulation and plant defense against abiotic stresses e.g. salinity. Quantification of the hormone concentration can reveal different plant strategies to cope with the stress, e.g. suppression of growth or mobilization of plant metabolism.

SA caused decrease in plant length, chlorophyll content, fresh weight of shoot and root of radish plants (Figures 2-6. This consequence may be related to the decrease of free IAA concentration (Figure 7 because SA can interfere with auxin responses resulting in stabilization of the Aux:IAA repressor proteins and inhibition of auxin responses (30). Furthurmore, (31) concluded that at SA treatment, auxin - induced ethylene production decreased more than 60% in hypocotyls of mung bean. Or because the significant decrease in GA concentration (Figure 8). This is compatible with (32) who showed that under salt stress, the endogenous GA and free SA content decreased, while a significant increase in the endogenous ABA and JA contents were observed. The results showed that salinity stress drasitically reduced growth and yield components of soybean by affecting endogenous growth hormones (32). This may be related to the ability of SA to counteract the inhibitory effects of several abiotic stress conditions during germination and seedling establishment, which is modulated by gibberellins, probably through a member of the GASA (Giberellic Acid Stimulated in Arabidopsis) gene family (33).

In spite of the reduction in IAA and GA concentrations, the internal free cytokinin and ABA concentrations in plants grown from primed and unprimed seeds did not change (Figure 9). This was compatible with the finding of (34) in pea plants. (35) found that the basal level of ABA and cytokinin appeared to play an important role in determining the response of a variety to salt stress and accumulation of the salt induced ABA and cytokinins , which isconcomitant with the changes in water potential and stomatal conductance of salt stressed plants.

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