

Impact of Salicylic Acid Seed Priming on Germination and the Combined Effect of Biochar and Salicylic Acid on Plant Nutrient Content under Salt Stress

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Abstract

In the initial phase of this study, the germination traits of maize seeds subjected to salicylic acid (SA) priming under saline stress were investigated. The treatments consisted of five SA concentrations: 0 (distilled water), 0.5, 0.75, 1.0, and 1.5 mM. In the subsequent phase, the experiment was extended to examine the combined effects of SA and biochar on the nutrient composition of maize plants exposed to salinity stress. Treatments included three concentrations of SA (0, 0.75, and 1.0 mM), three levels of biochar (0, 0.6, and 1.2%), and three salinity levels (0, 4, and 8 dS·m⁻¹) induced by NaCl. The findings revealed that under severe salinity stress (8 dS·m⁻¹), seed priming with SA at 0.5, 0.75, 1.0, and 1.5 mM improved germination rates by approximately 18%, 38%, 63%, and 88%, respectively, compared with the untreated control under the same salinity level. The elevated salinity markedly delayed germination and suppressed seedling growth; however, SA application extended the period between the first and the last germination while simultaneously improving seedling height. With increasing salinity, sodium and chloride accumulation in plant tissues rose significantly, while calcium and potassium concentrations declined. The combined application of 1.2% biochar and SA was particularly effective in reducing sodium buildup and preventing calcium depletion under saline conditions. Remarkably, at the highest salinity level, the simultaneous use of 1.5 mM SA and 1.2% biochar enhanced potassium content by more than 34% compared with the NaCl-only treatment (8 dS·m⁻¹ without SA or biochar). Moreover, phosphorus concentration in plant tissues increased by 20% and 44% under salinity levels of 4 and 8 dS·m⁻¹, respectively, relative to the non-saline control. Across all salinity treatments, SA consistently promoted phosphorus uptake compared with untreated plants.

Keywords: Germination, Sodium, Chloride, Foliar Spray, Biochar.

Introduction

Elevated soil salinity is widely acknowledged as a critical abiotic constraint that severely limits crop development and yield potential. The osmotic imbalance induced by excessive salt accumulation impairs cellular water uptake efficiency, subsequently interfering with essential metabolic pathways and physiological functions in plants [1,2]. Toxic elements such as sodium (Na^+) and chloride (Cl^-) accumulate in plant tissues under saline conditions, often competing with essential nutrients during uptake and ultimately leading to diminished crop yields [3]. Beyond its direct effects on plant health, salinity stress also deteriorates soil physical structure, alters its chemical properties, and disrupts ecological stability. Its consequences typically manifest as reduced agricultural output, decreased financial profitability, and increased soil degradation [4,5]. Among various physiological effects, salinity particularly impairs photosynthesis by reducing leaf expansion, chlorophyll content, and stomatal activity. Moreover, salinity stress adversely influences reproductive processes, including seed development, by inducing embryo abortion, programmed cell death, disruption in ovule formation, inhibition of stamen growth, and premature embryo senescence [6,7]. To address these challenges, seed priming has emerged as an effective, affordable, and practical strategy for improving seed performance under saline conditions. This technique enhances germination rates and promotes better seedling establishment in crop production systems [8,9]. Seed germination represents one of the most vital and sensitive phases in the life cycle of plants [10]. Numerous studies have demonstrated that priming treatments can significantly improve germination

indices of maize under saline conditions [11]. Salinity stress poses a considerable challenge to most crops, primarily due to elevated salt concentrations in the soil. Maize (*Zea mays* L.), ranking third globally among cereal crops after rice and wheat, is cultivated across diverse climates and soil types. Despite its adaptability, maize is considered moderately sensitive to salt stress [12]. This sensitivity is largely attributed to sodium ion accumulation in the leaf tissue. Moreover, salinity stress triggers oxidative damage in maize by promoting the overproduction of reactive oxygen species (ROS) within plant cells. While reclaiming saline soils typically demands long-term interventions, adopting both immediate and sustained management strategies is essential for improving productivity and enhancing farmers' livelihoods [13]. Salicylic acid (SA) plays a key role in regulating plant growth and development and is involved in different physiological processes, particularly in enhancing plant tolerance to environmental stresses. Research has shown that SA can lower the accumulation of sodium and chloride ions within plant cells. Furthermore, the exogenous application of salicylic acid increases its concentration inside plant tissues, leading to improved potassium uptake. Several studies have confirmed the positive effects of salicylic acid in boosting plant growth parameters under saline conditions [14,15]. In many arid and semi-arid regions, soils generally have low organic matter content, making the addition of organic amendments crucial for sustaining soil fertility and improving physical structure. Among these amendments, biochar has gained recognition for enhancing plant resilience to salinity. Biochar contributes to better soil water retention, enhances infiltration rates, and reduces the risk of erosion, thereby supporting crop production in challenging environments

[16]. The beneficial role of biochar in alleviating salinity stress is largely attributed to its ability to improve the physical and chemical characteristics of soil, enhance soil biological activity and physicochemical properties, and stimulate enzymatic processes within the soil [17-19]. The positive effect of biochar on plant performance under stress conditions has been confirmed across a range of plant species [20-22]. This investigation was structured to address two fundamental research aims: initially, to examine the influence of salicylic acid (SA) seed priming on germination parameters in *Zea mays* L. under NaCl-induced ionic stress; subsequently, to determine the potential synergistic effects of SA and pyrolyzed biomass (biochar) in mitigating NaCl phytotoxicity and enhancing mineral acquisition in developing maize plants.

Materials and Methods

Seed Germination Indices under the Effect of Salicylic Acid Pre-Treatment

To assess the germination traits of maize seeds subjected to salicylic acid (SA) priming under saline stress, a factorial experiment was conducted using a completely randomized design (CRD) with three replications. Initially, the seeds were surface-sterilized by immersion in a 3% sodium hypochlorite (NaOCl) solution for 30 seconds, followed by thorough rinsing five times with distilled water to remove any residual disinfectant. For priming, the disinfected seeds were soaked in prepared SA solutions at concentrations of 0 (distilled water), 0.5, 0.75, 1.5, and 2.0 mM. The seeds were incubated for 24 hours at 20 °C in complete darkness. After priming, 25 seeds from each treatment were transferred to sterile 9 cm Petri dishes lined with Whatman No. 1 filter paper. Each dish was supplied with 10 ml of

saline solution adjusted to three salinity levels: 0 (control), 4, and 8 dS·m⁻¹, prepared using NaCl. The Petri dishes were placed in a growth chamber set at 25 °C and maintained for five days. Germination was recorded daily for each replicate throughout the experimental period. Seed germination was monitored daily for each replicate throughout the five-day period. Seeds were classified as germinated when the radicle reached a length of at least 2 mm [23]. The germination percentage for each treatment was calculated using the formula provided by Maguire [24] (Equation 1).

$$\%G = \left(\frac{n}{N} \right) \times 100 \quad (1)$$

G: Germination efficiency (η)

Ratio of successfully germinated propagules (*n*) to total propagules incubated (*N*). Following a 120-hour incubation period, ten germinated specimens were aseptically extracted from each culture vessel. Morphometric analysis included measurement of epicotyl length from the hypocotyl-root junction to the apical meristem using precision calipers (resolution: 0.1 mm). Desiccated biomass quantification was performed via gravimetric analysis using an analytical balance (precision: 0.0001 g). Prior to mass determination, all plant material underwent complete dehydration in a forced-air drying oven maintained at 75°C ± 2°C for 48 hours to achieve constant mass.

The Effect of Salicylic Acid and Biochar on Plant Nutrient Content

A greenhouse experiment was conducted to evaluate the effects of biochar and salicylic acid (SA) on the nutrient composition of maize plants grown under salinity stress. The study followed a factorial arrangement within a

completely randomized design (CRD) with three replications. The experimental treatments comprised three concentrations of SA (0, 0.75, and 1.5 mM), three levels of biochar application (0, 0.6, and 1.2% by weight per pot), and three levels of salinity stress (0, 4, and 8 dS·m⁻¹) induced by sodium chloride (NaCl). The soil used in this experiment was collected from the research farm of the Department of Soil Science and Water Resources, Faculty of Agricultural Sciences, University of Baghdad. Following collection, the soil was transported to the greenhouse, where it was air-dried, sieved, and thoroughly homogenized before use in pot preparation and maize planting. The physical and chemical properties of the soil used in the experiment are presented in Table 1.

20 kg of soil was added to each pot. After preparing the soil in the pots and mixing it with specified amounts of biochar derived from date palm leaves and stems [25] (Table 2), the seeds used were of the hybrid maize variety Single Cross 704 (KSC704), obtained from the Agricultural Research Center (Aboqareeb Research Center - Seed Group). Five healthy seeds disinfected with Carboxin

Thiram were planted at a depth of 3 cm in each pot. After planting the seeds in the pots and the emergence of seedlings, at the three-leaf stage of seedling growth, thinning was performed, and only three healthy maize seedlings were retained in each pot for growth. Irrigation was uniformly applied to all pots according to field capacity until the different salinity levels were applied. Foliar spraying of the plants with salicylic acid was done until the entire leaf surface of each plant was wet and was repeated every two weeks. In the control pots, only distilled water was sprayed. Two days after the application of salicylic acid treatment, the salinity stress treatment began and continued until the end of the growing season. To apply salinity stress to the plants, NaCl salt was used. The pots were weighed every other day, and then the water deficit was replenished to field capacity plus an additional 15% as the leaching requirement. The plants were harvested four months after planting from the soil surface. After harvesting, the sodium and chloride content [26], as well as the calcium, phosphorus, nitrogen, and potassium content [27] of the plants were measured.

Table 1 Physical and chemical analyze of soil using in the experiment

Particle density (%)	Bulk Density (g·cm ⁻³)	CEC (meq.100g ⁻¹)	CO ₃ ²⁻ (g.kg ⁻¹ soil)	CaSO ₄ (%)	O.M (%)	EC (dS.m ⁻¹)	PH	
24	1.45	14.8	136.5	0.64	3.15	2.66	7.41	
Clay (%)	Silt (%)	Sand (%)	©K	©P	©N	PWP(%)		
38	40	22	163	9.0	56	12		
*NO ₃ ⁻	*CO ₃ ²⁻	*HCO ₃ ⁻	*SO ₄ ²⁻	*Cl ⁻	*K ⁺	*Mg ²⁺	*Na ⁺	*Ca ²⁺
21	35	608	209	691	25	248	677	586

* mg. l⁻¹; © PPM.

Table 2 Characteristic of biochar using in the experiment

Mg (mg. g ⁻¹)	Ca (mg. g ⁻¹)	Na (mg. g ⁻¹)	K (mg. g ⁻¹)	P (mg. g ⁻¹)	C: N	N (mg. g ⁻¹)	C (mg. g ⁻¹)	PH	EC 1:5 (ds.m ⁻¹)
0.8	7.43	0.5	7.8	1.6	69.6	4.6	320	7.30	3.21

Selection of Salicylic Acid Concentrations and Monitoring of Phytotoxic Effects

The concentration range of salicylic acid (SA) used in this study (0, 0.5, 0.75, 1.0, 1.5, and 2 mM) was determined based on a combination of literature benchmarks and preliminary laboratory trials. Previous studies have reported that low to moderate SA concentrations can enhance seed germination and stress tolerance in maize and other crops under saline conditions [23]. Preliminary experiments confirmed that concentrations above 2 mM could inhibit germination and seedling growth, while concentrations within the 0.5–1.5 mM range improved germination and early seedling development without observable toxicity. During the experimental period, seedlings treated with the highest concentration (2 mM) were closely monitored for potential phytotoxic effects, including leaf chlorosis, necrosis, stunted growth, or abnormal morphology. No significant phytotoxicity was observed during the germination and early seedling growth periods. However, extended preliminary trials indicated that concentrations above 2 mM could cause mild growth inhibition, which justified selecting 1.5 mM as the upper treatment level in the main experiment.

Biochar Characterization and Application

The biochar used in this experiment was chemically characterized to ensure its functional properties relevant to ion regulation under salinity stress. The cation exchange capacity (CEC) was determined using the ammonium acetate method at pH 7, which provides an estimate of the biochar's ability to retain essential cations (*e.g.*, K^+ , Ca^{2+} , and Mg^{2+})

and reduce the bioavailability of toxic sodium ions. Additionally, the surface area and porosity of the biochar were measured using the Brunauer–Emmett–Teller (BET) method. A higher surface area enhances nutrient adsorption, water retention, and microbial colonization, all of which can contribute to improved ion homeostasis and plant growth under saline conditions. Biochar was applied at three levels (0%, 0.6%, and 1.2% by weight per pot) and thoroughly mixed into the soil to ensure uniform distribution, thereby maximizing its effectiveness in mitigating the negative impacts of salinity on maize plants.

Statistical Analysis

The statistical analysis of the research data was conducted using JMP-8 software. The comparison of mean data was also conducted using the LSD test.

Results and Discussion

Seed Germination Indices under the Effect of Salicylic Acid Pre-treatment

The results of the study showed that the germination percentage ($P \leq 0.01$), the number of days to the first and last seed germination, and the seedling height were significantly affected by the experimental treatments ($P \leq 0.01$).

The exposure to salinity stress caused a significant decline in the germination percentage of maize seeds. Nevertheless, applying salicylic acid under saline conditions had a beneficial effect by enhancing seed germination rates. Across various levels of salinity stress, different concentrations of salicylic acid consistently improved germination percentages compared to the control treatment without salicylic acid (Table 3).

Table 3 Analysis of variance of seed germination traits under different treatment

Source of variation	df	Germination	Days to the first day of germination	Days to the last day of germination	Plant height	Seed dry weight
Salinity	2	14441**	2.26**	2.23**	15.41**	0.003 ^{ns}
SA	5	344**	10.93**	10.35**	13.82**	0.001 ^{ns}
Salinity × SA	10	103**	0.04 ^{ns}	0.04 ^{ns}	0.36 ^{ns}	0.001 ^{ns}
Error	36	7.65	0.07	0.06	0.19	0.001

ns: Indicates non-significant treatment effects ($p > 0.05$); *: Denotes statistical significance at the 5% probability threshold ($p \leq 0.05$); **: Represents highly significant effects at the 1%.

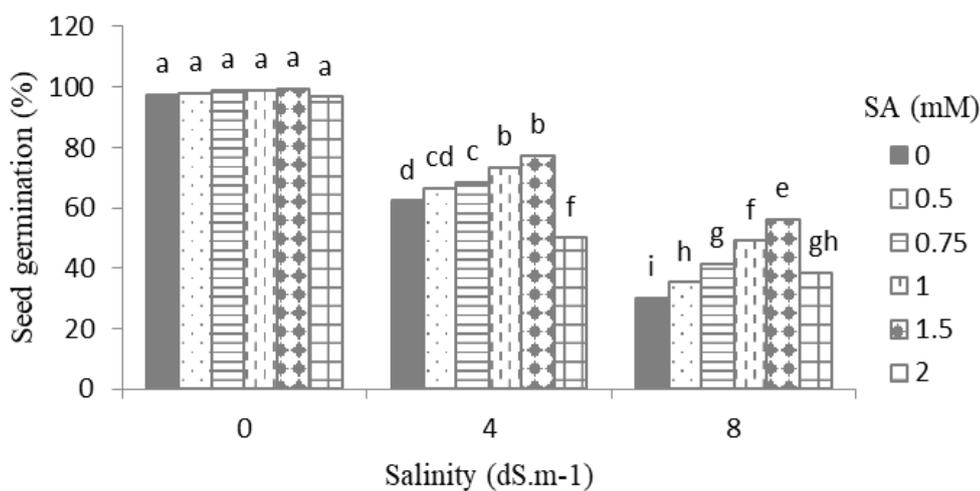


Figure 1 Effect of salinity levels (0, 4, and 8 dS·m⁻¹ NaCl) and salicylic acid (SA) concentrations (0, 0.5, 0.75, 1.0, and 1.5 mM) on maize seed germination percentage. Data represent mean ± standard error (n = 3). Bars sharing the same letter within each salinity level are not significantly different at $p \leq 0.05$ according to the LSD test

Specifically, at a salinity level of 4 dS·m⁻¹, salicylic acid concentrations of 0.5%, 0.75%, 1.0%, and 1.5% resulted in approximately 10%, 10%, 18%, and 24% increases in seed germination, respectively, relative to the control under the same salinity condition.

At a higher salinity level of 8 dS·m⁻¹, these same SA concentrations enhanced germination by roughly 18%, 38%, 63%, and 88%, respectively, compared to untreated seeds at the equivalent stress level (see Figure 1).

The experimental findings revealed that elevating salinity stress to 8 dS·m⁻¹ resulted in a 42% prolongation of the initial seed germination period relative to the control group (Table 4).

Additionally, exogenous salicylic acid application extended the germination duration (Table 5). Data analysis demonstrated that heightened salinity concentrations accelerated the final germination phase (Table 4), while salicylic acid treatment delayed its completion (Table 5). Further observations indicated that saline conditions adversely affected hypocotyl elongation, with an 8 dS·m⁻¹ treatment causing a 12.3% reduction in seedling height compared to non-saline controls (Table 4). In contrast, salicylic acid supplementation exhibited a positive regulatory effect on maize seedling growth, enhancing vertical development (Table 5).

Table 4 Effect of salinity on days to the first and the last days of germination and plant height

Salinity (ds.m ⁻¹)	Days to the first day of germination	Days to the last day of germination	Plant height (cm)
0	1.67	3.53	13.98
4	1.92	3.08	13.69
8	2.37	2.84	12.25

Within each column, means annotated with identical superscript letters exhibit no statistically significant divergence at $\alpha = 0.05$, as determined by Fisher's Least Significant Difference (LSD) post hoc analysis.

Table 5 Effect of SA on days to the first and the last days of germination and plant height

SA (mM)	Days to the first day of germination	Days to the last day of germination	Plant height (cm)
0	0.83	2.07	11.62
0.5	1.00	2.25	12.15
0.75	1.26	2.53	13.41
1	2.43	3.35	12.42
1.5	2.96	3.88	14.64
2	3.43	4.83	14.64

Within each column, means annotated with identical superscript letters exhibit no statistically significant divergence at $\alpha = 0.05$, as determined by Fisher's Least Significant Difference (LSD) post hoc analysis.

The reduction in seed germination percentage following the application of salinity stress in plants is due to the decrease in water potential and the toxicity caused by the excessive accumulation of sodium and chloride ions, which subsequently reduces essential growth ions such as calcium and potassium. In general, salinity inhibits the release of food resources for seed consumption, leading to a decrease in germination percentage, germination rate, and seedling growth [28]. Consistent with the results of this study, the reduction in seed germination indices such as percentage and germination rate under salinity stress has been reported in other studies [29]. Salinity in plants reduces lipid breakdown and substance production in the plant. Consequently, with the reduction in the activity of certain enzymes and disruption of the plant photosynthesis process, nitrogen and carbon metabolism in the plant are disturbed, resulting in reduced cell division and plant growth [30,31]. In general, the inhibitory effects of salinity

on the photosynthesis process and biochemical reactions in the plant lead to reduced seedling growth [32]. In this study, salinity application also reduced maize seedling height. The reduction in plant growth in maize under salinity stress has been reported in other studies [33]. Salicylic acid is known as a germination stimulant [28]. The germination percentage of seeds under salicylic acid pre-treatment is due to the positive effect of this substance on the plant's antioxidant system and the reduction of salinity toxicity [34,35]. Additionally, salicylic acid is effective in improving seedling growth and increasing its height under salinity conditions by preventing auxin oxidation. This compound prevents the reduction of cytokinins by altering hormonal balance under salinity conditions and increasing auxin and abscisic acid. The increase in abscisic acid concentration is effective in improving anti-stress reactions in seedlings [33,36]. The positive effect of salicylic acid in improving seed

germination under salinity stress has also been reported in other studies [29].

The Effect of Salicylic Acid and Biochar on Plant Nutrient Content

The analysis of variance for nutrient concentrations in maize plants revealed that foliar application of salicylic acid (SA) and the incorporation of biochar significantly influenced elemental composition (Table 6).

Increasing salinity levels led to a marked rise in sodium content in plant tissues compared with the control treatment (0 dS·m⁻¹ NaCl + 0 mM SA + 0% biochar). The highest sodium accumulation was recorded under the treatments 8 dS·m⁻¹ NaCl + 1.5 mM SA + 0.6% biochar, 8 dS·m⁻¹ NaCl + 0.75 mM SA + 0.6% biochar, and 8 dS·m⁻¹ NaCl + 0 mM SA + 0.6% biochar (Table 7). However, across all salinity levels, the application of the highest biochar dose (1.2%), in combination with different SA concentrations, effectively mitigated excessive sodium buildup in plant tissues compared with the corresponding saline controls (Table 7).

Regarding calcium concentration, results indicated that increasing salinity stress significantly reduced calcium content in plant tissues, with the lowest levels observed under the highest salinity (8 dS·m⁻¹). Nevertheless, the combined application of 1.2% biochar and 1.5 mM SA significantly alleviated calcium depletion compared with the respective saline control at 4 dS·m⁻¹. Furthermore, even under non-saline conditions, the combined use of biochar and SA improved calcium concentrations relative to the untreated control. The highest calcium level was observed in the treatment 0 dS·m⁻¹ NaCl + 1.5 mM SA + 1.2% biochar (Table 7).

Salinity stress significantly increased chlorine accumulation in maize plants

compared with the control treatment. The highest chlorine concentrations were observed in the treatments 8 dS·m⁻¹ NaCl + 0 mM SA + 0.6% biochar and 4 dS·m⁻¹ NaCl + 0 mM SA + 0.6% biochar, showing increases of 29.4% and 30%, respectively, relative to the control (Table 7).

Analysis of potassium concentration revealed that salinity stress markedly reduced potassium levels in plant tissues. At the highest salinity (8 dS·m⁻¹ NaCl), the combined application of 1.5 mM SA and 1.2% biochar increased potassium content by more than 34% compared with 8 dS·m⁻¹ NaCl + 0 mM SA + 0% biochar. Similarly, at 4 dS·m⁻¹ NaCl, the same treatment combination enhanced potassium levels by more than 20% compared with 4 dS·m⁻¹ NaCl + 0 mM SA + 0% biochar. The maximum potassium concentration overall was recorded in plants grown under non-saline conditions with the treatment 0 dS·m⁻¹ NaCl + 1.5 mM SA + 1.2% biochar (Table 7).

Phosphorus content in plant tissues also increased significantly under salinity stress, showing rises of 20% and 44% at 4 and 8 dS·m⁻¹ NaCl, respectively, compared with the control. Across all salinity levels, SA application at different concentrations consistently enhanced phosphorus uptake compared with untreated plants. The highest phosphorus concentration was observed under 0 dS·m⁻¹ NaCl + 0.75 mM SA, whereas the lowest concentration occurred in the absolute control (0 dS·m⁻¹ NaCl + 0 mM SA) (Figure 2).

Application of biochar at a salinity level of 6 dS·m⁻¹ effectively inhibited a marked rise in the sodium content within the plant tissues. Moreover, incorporating biochar into the soil led to a reduction in chloride accumulation in plants experiencing salinity stress.

Table 6 Analysis of variance of Na, Ca, Cl, K, P, and N concentrations under different treatments

S.O. V	df	Na	Ca	Cl	K	P	N
Salinity (S)	2	0.36**	0.91**	1.05**	3.21**	0.0001 ^{ns}	0.09 ^{ns}
SA	2	0.009**	0.02**	0.04**	0.42**	0.007 ^{ns}	0.02 ^{ns}
Biochar (B)	2	0.002**	0.001 ^{ns}	0.10**	0.09**	0.006 ^{ns}	0.012 ^{ns}
SA×S	4	0.006**	0.004**	0.04**	0.04**	0.021*	0.057 ^{ns}
SA×B	4	0.0002 ^{ns}	0.003*	0.06**	0.007**	0.008 ^{ns}	0.090 ^{ns}
S×B	4	0.001**	0.001 ^{ns}	0.08**	0.01**	0.007 ^{ns}	0.077 ^{ns}
SA×S×B	8	0.001**	0.002*	0.04**	0.004**	0.011 ^{ns}	0.070 ^{ns}
Error	54	0.0002	0.0009	0.001	0.001	0.007	0.064

ns: Indicates non-significant treatment effects ($p > 0.05$); *: Denotes statistical significance at the 5% probability threshold ($p \leq 0.05$); **: Represents highly significant effects at the 1%.

Table 7 Different concentrations of salicylic acid and biochar on plant height and shoot and root dry weight under saline condition

Salinity (dS.m ⁻¹)	SA (mM)	Biochar (%)	Na (mg.g ⁻¹ DW)	Ca (mg.g ⁻¹ DW)	Cl (mg.g ⁻¹ DW)	K (mg.g ⁻¹ DW)
0	0	0	0.39 ^{no}	0.85 ^{de}	1.63 ^g	2.20 ^f
		0.6	0.38 ^o	0.86 ^{cde}	1.43 ⁱ	2.26 ^{def}
		1.2	0.40 ^{mno}	0.87 ^{bcd}	1.53 ^h	2.27 ^{cd}
		0	0.39 ^{mno}	0.88 ^{bcd}	1.46 ^j	2.33 ^{bc}
		0.6	0.41 ^{lmn}	0.89 ^{bcd}	1.42 ^j	2.34 ^b
		1.2	0.42 ^{klm}	0.84 ^{de}	1.35 ^k	2.38 ^b
	0.75	0	0.41 ^{k-n}	0.91 ^{abc}	1.42 ^j	2.34 ^b
		0.6	0.43 ^{ijk}	0.92 ^{ab}	1.47 ^{ij}	2.37 ^b
		1.2	0.43 ^{jkl}	0.95 ^a	1.52 ^{hi}	2.44 ^a
		0	0.45 ^{ij}	0.66 ^h	1.46 ^j	1.88 ⁱ
		0.6	0.45 ^{ij}	0.66 ^h	2.12 ^a	2.06 ^{gh}
		1.2	0.43 ^{jkl}	0.73 ^{fg}	1.77 ^{ef}	2.02 ^{hi}
4	0.75	0	0.46 ^{hi}	0.73 ^{fg}	1.83 ^d	2.21 ^{ef}
		0.6	0.48 ^{ghi}	0.68 ^{gh}	1.94 ^b	2.22 ^{def}
		1.2	0.45 ^{ij}	0.65 ^h	1.84 ^d	2.26 ^{def}
		0	0.50 ^g	0.75 ^f	1.64 ^g	2.11 ^g
		0.6	0.53 ^f	0.74 ^f	1.74 ^f	2.27 ^{cde}
		1.2	0.56 ^e	0.81 ^e	1.92 ^b	2.27 ^{cd}
	1.5	0	0.61 ^{cd}	0.49 ^j	1.72 ^f	1.48 ^{no}
		0.6	0.65 ^a	0.51 ^{ij}	2.11 ^a	1.46 ^o
		1.2	0.64 ^{ab}	0.52 ^{ij}	1.91 ^{bc}	1.52 ^{mn}
		0	0.63 ^{bc}	0.51 ^{ij}	1.81 ^{de}	1.56 ^{lm}
		0.6	0.68 ^a	0.52 ^{ij}	1.76 ^f	1.59 ^l
		1.2	0.64 ^{ab}	0.54 ⁱ	1.63 ^g	1.77 ^k
8	0.75	0	0.62 ^{bc}	0.52 ^{ij}	1.77 ^{ef}	1.74 ^k
		0.6	0.66 ^a	0.54 ⁱ	1.86 ^{cd}	1.80 ^k
		1.2	0.59 ^{de}	0.51 ^{ij}	1.74 ^f	1.99 ⁱ

Within each column, means annotated with identical superscript letters exhibit no statistically significant divergence at $\alpha = 0.05$, as determined by Fisher's Least Significant Difference (LSD) post hoc analysis.

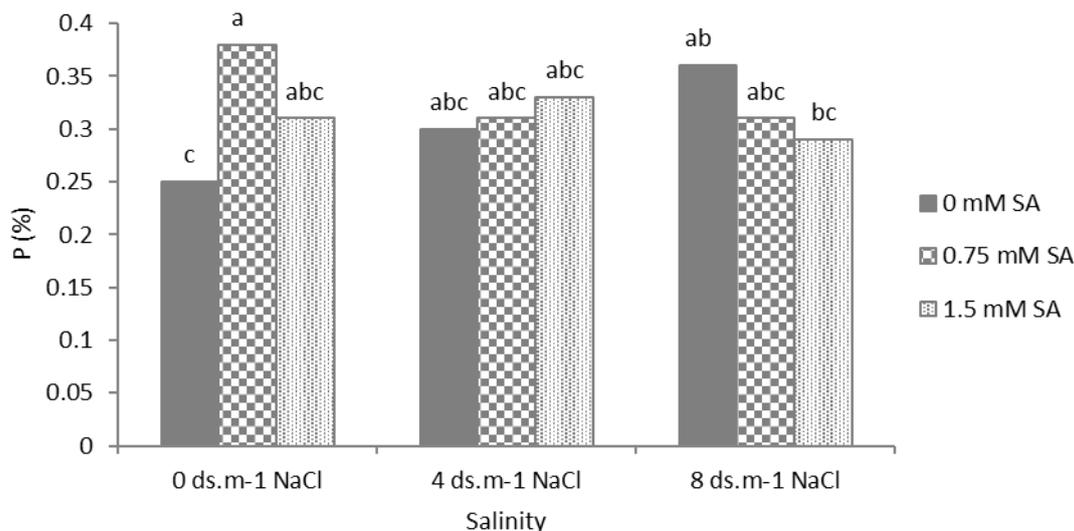


Figure 2 Effect of salinity levels (0, 4, and 8 dS·m⁻¹ NaCl) and salicylic acid (SA) concentrations (0, 0.75, and 1.5 mM) on phosphorus (P) concentration in maize plants. Data are presented as mean ± standard error (n = 3). Different letters above the bars indicate statistically significant differences among treatments based on the LSD test at p ≤ 0.05

The ion exchange process triggered by biochar, when used as an organic soil amendment [14], plays a crucial role in lowering the levels of harmful sodium and chloride ions absorbed by the plant. Enhancement of plant growth parameters, including the dry biomass of both shoots and roots under saline conditions, is closely linked to the improved photosynthetic efficiency promoted by salicylic acid. These findings align with previous research demonstrating that salicylic acid application mitigates the adverse effects of salinity stress and boosts growth indicators in various plant species [37]. The beneficial effect of salicylic acid on enhancing grain yield is attributed to its role in promoting the translocation of assimilates to the grains during the grain filling period. The previous studies have documented improvements in the growth of barley and maize under salinity stress following salicylic acid application [38,39]. It has been emphasized [40] that salicylic acid positively influences the uptake of calcium and potassium ions while

limiting sodium and chloride absorption in wheat exposed to saline conditions. Similarly, the current study found that foliar application of salicylic acid led to increased levels of calcium, potassium, and phosphorus in maize plants. Modifications in ion uptake patterns induced by salicylic acid under salt stress represent one of the adaptive mechanisms plants use to mitigate salinity damage [41]. Moreover, it has been reported elevated potassium and calcium concentrations in safflower grown under saline conditions [42]. The present study demonstrated that salinity stress negatively affected maize seed germination, seedling growth, and nutrient accumulation, consistent with previous reports indicating that high Na⁺ and Cl⁻ concentrations disrupt water uptake, ionic balance, and enzymatic activities in plants [43]. However, the application of salicylic acid (SA) significantly alleviated the adverse effects of salinity, improving germination percentage, seedling height, and nutrient content. Mechanistically, SA may enhance osmotic adjustment by promoting the

synthesis of osmolytes and antioxidant enzymes, thereby reducing oxidative damage induced by salt stress [44,45]. Additionally, SA likely modulates ion transport, contributing to lower sodium accumulation and higher potassium and calcium levels in plant tissues, as observed in our results. The combined application of biochar with SA further amplifies these benefits, possibly by improving soil cation exchange capacity (CEC), retaining essential nutrients, and reducing the bioavailability of toxic ions such as Na^+ and Cl^- . The observed increase in phosphorus uptake under SA treatments could be attributed to enhanced root growth and improved nutrient solubilization in the rhizosphere, while biochar may also act as a reservoir facilitating sustained nutrient availability [46]. These mechanistic insights, drawn from our experimental data and supported by previous studies, provide a physiological basis for the mitigation of salinity stress. Furthermore, the synergistic effects of SA and biochar highlight their potential as practical strategies to improve crop performance under saline conditions, extending beyond mere confirmation of literature findings to offer new applied perspectives [20,47].

Possible Mechanisms of Salicylic Acid on Germination Dynamics

Salicylic acid (SA) has a dual effect on seed germination dynamics: it can delay the timing of germination events while simultaneously enhancing the overall germination percentage. This seemingly paradoxical effect can be explained through several physiological and biochemical mechanisms.

Modulation of Reactive Oxygen Species (ROS) and Antioxidant Systems

SA is known to regulate the production of ROS, which act as signaling molecules during seed germination. Moderate ROS levels are required for breaking dormancy and promoting germination, but excessive ROS can damage cellular components. SA treatment may temporarily slow germination by fine-tuning ROS levels, preventing oxidative stress during early imbibition while allowing seeds to survive under saline conditions. The delay ensures that germination occurs under more favorable intracellular conditions, ultimately improving the final germination percentage [48].

Osmotic Adjustment and Stress Priming

Under salinity stress, SA can enhance osmolyte accumulation (such as proline and soluble sugars) and stabilize cellular membranes. This osmotic adjustment may slow the initial radicle emergence (delaying first germination events) but prepares the seed to tolerate ionic and osmotic stress, increasing the likelihood of successful germination over time [49].

Hormonal Crosstalk and Dormancy Regulation

SA interacts with other phytohormones such as abscisic acid (ABA) and gibberellins (GA), which are critical for germination timing. SA can transiently enhance ABA signaling or modulate GA responsiveness, causing a delay in radicle protrusion. However, as stress mitigation mechanisms are activated, GA-driven germination resumes, resulting in higher final germination percentages compared to untreated stressed seeds [50,51].

Enhanced Repair and Metabolic Activation

SA may slow germination initially by promoting energy allocation toward cellular repair and the activation of

metabolic pathways necessary under stress. This ensures that only viable seeds complete germination, leading to an improved overall success rate [50,51].

Practical Implications of SA-Biochar Application

Based on the results of this study, the combined application of salicylic acid (SA) and biochar demonstrates significant potential for improving maize performance under saline conditions. Biochar, incorporated into the soil pre-planting, provides a sustained improvement in soil properties, including enhanced cation exchange capacity (CEC), water retention, and nutrient availability. These long-term benefits help mitigate sodium accumulation and support nutrient uptake throughout the growth period. SA, when applied either as a pre-planting seed priming agent or as a foliar treatment, enhances stress tolerance by modulating antioxidant activity, osmotic adjustment, and ion homeostasis. In this study, seed priming with SA delayed early germination slightly, but ultimately increased final germination percentages and seedling vigor, suggesting that pre-planting application effectively prepares seeds for saline stress [52,53].

Therefore, for saline fields, a combined strategy is recommended: Biochar incorporation pre-planting to improve soil physical and chemical properties. SA seed priming before sowing enhances germination and early seedling stress tolerance. Mid-season foliar SA applications could serve as a complementary approach to sustain stress mitigation during critical growth stages, particularly if salinity intensifies later in the season. However, the most consistent benefits observed in this study arose from pre-planting interventions, as they provide both soil-mediated and

seed-mediated protection against salinity stress [52,53].

Conclusion

The results showed that applying salinity stress led to a reduction in seed germination indices and changes in the nutrient content of the plant. In the first phase, seed priming with salicylic acid improved the seed germination percentage and seedling growth height. In the second phase of the experiment, the use of biochar and salicylic acid was effective in adjusting the nutrient content under salinity conditions. Overall, it can be mentioned that seed priming with salicylic acid, as well as foliar application of it and the use of biochar in the soil, is effective in improving plant growth under salinity conditions.

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