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The effect of salicylic acid and brassinosteroids on the performance of sweet pepper plant (*Capsicum annuum*) under different salinity levels

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ABSTRACT

A study was carried out at the Faculty of Agriculture and Veterinary Medicine's experimental station to assess the effect of different concentrations of salicylic acid and brassinosteroid on pepper plants under different salinity levels in greenhouse conditions. Three salicylic acid (SA) and brassinosteroid (BR) concentrations (0, 0.05, and 0.5 mM) and three NaCl concentrations (0, 50, and 150 mM) were used. The experiment was set up as a split-plot with three replicates. Both BR and SA had a similar positive effect on fruit number per plant, total yield per plant, and SPAD, fresh and dry weight of the plant, as well as fresh and dry weight of the root. BR had no effect on plant height, whereas SA at 0.05 mM increased plant height significantly. BR had no effect on root/shoot ratio, whereas SA at 0.5 mM significantly increased root/shoot ratio. The study found that using brassinosteroid and salicylic acid reduced the effect of salinity on pepper plant yield and growth.

Keywords: Brassionosteroid; Salicylic acid; Salinity; SPAD; Sweet pepper

1. Introduction

Salinity and drought are considered two of the main abiotic stresses that affect plants and cause a reduction in plant growth and productivity. Various salt-degraded soils cover approximately 6% of the world's total land area and 23% of cultivated land [1].

Salinity led to a reduction in processes associated with plant growth, development, and productivity [2,3]. Palestine is classified as arid and semi-arid region. Water scarcity is a major limiting factor for optimal agricultural production in Palestine, exacerbating the salinity problem. Furthermore, the intensive cropping system hastens soil deterioration and land degradation caused soil salinization mainly in the Jordan valley [4].

Pepper (*Capsicum annuum* L.) is one of the important vegetables cultivated in Palestine and it is grown under greenhouse conditions during temperate periods and the open field during warm periods [4]. Sweet peppers are used in salads, cooked dishes and have high nutritional values as they contain a high level of vitamin A and vitamin C. Pepper plants are considered moderately sensitive, susceptible to salt stress. Soil salinity, if not appropriately managed, may become a limiting factor for pepper growth and productivity [5]. Moreover, many crop plants are susceptible and cannot survive under conditions of high salinity or can survive but with decreased yields. Pepper is among crops that are susceptible for salinity, the production may be reduced up to 14% for every unit increase of salinity above the threshold value [6]. Various management practices were implemented to address salinity stress. Plant growth

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regulators were also used to enhance plant growth under salinity stress, among these are salicylic acid (SA) and brassinosteroid, promote growth and alleviate salinity stress (BRs). Salicylic acid (SA) is a hormone-like substance that influences photosynthetic rate [7]. Many plants can benefit from the application of SA externally [8,9]. Brassinosteroids have been shown to form a wide range of morphological and physiological responses in plants, including the reduction of abiotic stresses like salinity [5,10–12].

Therefore, the main objective of this study is to evaluate the effect of both salicylic acid (SA) and brassinosteroids (BRs) on pepper growth and productivity under saline conditions.

2. Materials and methods

2.1. Plant material and experiment site

The experiment was carried out in greenhouse conditions at An-Najah National University, Faculty of Agriculture and Vet. Med. Palestine (32.31519°N, 35.02033°E, 75 m altitude). Pepper seedlings were grown in plastic pots (10 L) (one seedling per pot) filled with sand and peat-moss mixture (1:1, v/v) during the 2018–2019 growing season. Seedlings were grown in the greenhouse under natural light conditions. One month after transplanting, the effect of salinity, BRs, and SA was investigated by applying three salinity levels (0, 50, 150 mM NaCl) and three levels of BRs and SA (0, 0.05, and 0.5 mM).

2.2. Salinity, BR, and SA preparation

For BR, 24-epibrassinolide ($C_{28}H_{48}O_6$, molecular weight = 480; Sigma) was used, and for SA (2 hydroxybenzoic acid, 2^{-}(HO) $C_6H_4CO_2H$, molecular weight = 138.12; Sigma) were used. For stock preparation, each hormone was initially dissolved in 1 ml ethanol and three concentrations were prepared (0, 0.05, and 0.5 mM) using distilled water containing 0.02% Tween 20 (polyoxyethylenesorbitan monolaurate; Sigma Chemicals, UK) as a surfactant.

Three concentrations of NaCl were used (0, 50 and 150 mM) (Table 1). Pots were irrigated manually according to crop water requirement. The combination of each hormone and NaCl concentrations were considered the experiment's treatment; the treatments were arranged in a completely randomized design with three replicates for each treatment.

2.3. Growth, yield and yield components

During the growing season the following parameters were evaluated:

- *Fruit number*: Total number of fruit per plant was counted.
- *Fruit weight*: Total number of fruit per plant was weighed.
- *Chlorophyll contents*: The chlorophyll readings and leaf greenness of the pepper plants were taken using a chlorophyll meter (chlorophyll meter SPAD-502 Plus, Konica Minolta sensing, Inc. Japan).

At maturity the following parameters were evaluated:

- *Plant height*: from the soil surface to the tip of plants.
- *Fresh and dry weight*: The total fresh and dry weight of above-ground biomass and root biomass per plant was weighed directly at the end of the growing season. Dry weights per plant were weighed after oven-dried at 70°C for 48 h.
- *Root/shoot (R/S) ratio*: The R/S ratio was calculated by dividing roots dry weight/shoot dry weight.

2.4. Statistical analysis

To assess the differences between treatment means, Minitab software was used to run a factorial ANOVA test and a Tukey Multiple Range Test at a 5% probability level. Each hormone in relation to salinity was analyzed independently.

3. Results

3.1. Effect of salicylic acid and brassinosteroid on yield and yield components.

The result showed that salinity has high significant negative effect on the fruit number of peppers, when salinity level increases the fruit number decrease (Table 2). In addition, SA and BR treatments have a significant positive effect on the fruit number (Table 2). Both BR and SA showed a similar positive effect on fruit number per plant. Fruit numbers ranged between 19.67 and 21.22 under BR treatment (0.5 and 0.05 mM respectively) and 19.67 to 24.56 under SA treatment (0.5 and 0.05 mM respectively).

No significant interaction effect of BR on fruit number whereas SA showed a significant interaction effect (Table 3). 50 mM NaCl + 0.05 mM SA showed a higher

Table 1
Treatments used in the experiment

Number	Treatment
1	0 mM NaCl + 0 mM hormone
2	0 mM NaCl + 0.05 mM BR
3	0 mM NaCl + 0.5 mM BR
4	0 mM NaCl + 0.05 mM SA
5	0 mM NaCl + 0.5 mM SA
6	50 mM NaCl + 0 mM BR
7	50 mM NaCl + 0.05 mM BR
8	50 mM NaCl + 005 mM BR
9	150 mM NaCl + 0 mM BR
10	150 mM NaCl + 0.05 mM BR
11	150 mM NaCl + 005 mM BR
12	50 mM NaCl + 0 mM SA
13	50 mM NaCl + 0.05 mM SA
14	50 mM NaCl + 005 mM SA
15	150 mM NaCl + 0 mM SA
16	150 mM NaCl + 0.05 mM SA
17	150 mM NaCl + 005 mM SA

Table 2
Main effect of NaCl treatments, Brassionosteroid (BR) and salicylic acid (SA) on plant height (cm), fruit number per plant and yield (g/plant) parameters of sweet pepper

Main factor		Plant height (cm)		Fruit number		Yield (g/plant)	
		BR	SA	BR	SA	BR	SA
NaCl treatments	NaCl Control	78.22^{a}	88.78^{a}	28.56^{a}	29.56^{a}	1,686.01^{a}	1,801.44^{a}
	50 mM	44.78^{b}	45.78^{b}	18.33^{b}	20.67^{b}	382.83^{b}	387.70^{b}
	150 mM	31.11^{c}	31.56^{c}	2.67^{c}	2.67^{c}	51.20^{c}	42.76^{c}
	p-value	***	***	***	***	***	***
Hormone treatment	Hormone Control	47.33^{a}	47.33^{b}	8.67^{b}	8.67^{b}	437.64^{b}	437.64^{b}
	0.05 mM	53.44^{a}	62.33^{a}	21.22^{a}	24.56^{a}	800.88^{a}	984.11^{a}
	0.5 mM	53.33^{a}	56.44^{ab}	19.67^{a}	19.67^{a}	881.52^{a}	810.14^{a}
	p-value	NS	**	**	***	***	***

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

Table 3
Interaction effects of NaCl treatments, Brassionosteroid (BR) and salicylic acid (SA) on plant height (cm), fruit number per plant and yield (g/plant) parameters of sweet pepper

NaCl × Hormone	Plant height (cm)		Fruit number		Yield (g/plant)	
	BR	SA	BR	SA	BR	SA
0 mM × 0 mM	75.67	75.67^{bc}	17.33	17.33^{bc}	1,088.27^{c}	1,088.27^{b}
0 mM × 0.05 mM	78.00	101.67^{a}	32.00	35.67^{a}	1,783.57^{b}	2,337.30^{a}
0 mM × 0.5 mM	81.00	89.00^{ab}	36.33	35.67^{a}	2,186.20^{a}	1,978.77^{a}
50 mM × 0 mM	35.67	35.67^{de}	7.33	7.33^{bcd}	196.57^{ef}	196.57^{c}
50 mM × 0.05 mM	51.67	56.67^{cd}	28.67	34.67^{a}	561.43^{d}	560.60^{bc}
50 mM × 0.5 mM	47.00	45.00^{de}	19.00	20.00^{b}	390.50^{de}	405.93^{bc}
150 mM × 0 mM	30.67	30.67^{e}	1.33	1.33^{d}	28.10^{f}	28.10^{c}
150 mM × 0.05 mM	30.67	28.67^{e}	3.00	3.33^{cd}	57.63^{f}	54.43^{c}
150 mM × 0.5 mM	32.00	35.33^{de}	3.67	3.33^{cd}	67.87^{f}	45.73^{c}
p-value	NS	*	NS	**	***	**

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

fruit number per plant (34.67 fruit per plant) which was significantly higher than the control treatment (17.33 fruits per plant) (Table 3). The results showed that salinity caused a significant reduction in total plant yield (Table 2). Both BR and SA significantly improved total yield per plant. Both hormones showed significant interaction with NaCl treatments (Table 3). Total plant yield was significantly higher under 0.5 mM BR than the control whereas both SA concentrations showed a significant difference than the control. A clear interaction effect was exhibited for both hormone under the 0 NaCl on the yield, SA improved the yield at the 0.5 mM level while BR improved the yield at 0.05 mM level. However, none of the used concentrations of BR and SA showed significant improvement of total plant yield under both NaCl levels (50 and 150 mM).

3.2. Effect of salicylic acid and brassinosteroid on plant height

A significant negative effect of salinity on plant height was observed (Table 2). BR did not significantly affect plant height whereas SA at 0.05 mM significantly increased plant height (Table 2). Plant height ranged from 47.33 to 53.44 cm under BR treatment and from 47.33 to 62.33 cm under SA treatments. No significant interaction effect of BR on plant height whereas SA showed a significant interaction effect. Applying SA at 0.05 or 0.5 mM significantly increased plant height (101.67 and 89.00 cm respectively) than the control treatment (75.67 cm).

3.3. Effect of salicylic acid and brassinosteroid on chlorophyll content

The results showed that salinity caused a significant reduction of SPAD (chlorophyll content) (Table 4). Under salinity treatments, SPAD ranged from 41.09 to 59.97. Both BR and SA significantly improved SPAD per plant. Different BR and SA concentrations showed similar effects without any significant difference (Table 4). SPAD ranged from 42.07 to 58.80 under BR treatments and from 42.07 to 58.67 under SA treatment. No significant interaction was observed between BR treatments and NaCl concentrations

whereas significant interaction was observed between SA and NaCl treatments (Table 5). SA at 0.05 and 0.5 improved SPAD significantly at 50 mM NaCl (Table 5).

3.4. Effect of salicylic acid and brassinosteroid on plant fresh and dry weight

Significant negative effect of salinity, BR and SA on plant fresh weight was observed (Table 4). Plant fresh weight ranged from 198.51 to 369.42 g under BR treatment and from 217.48 to 365.53 g under SA treatment. Both used concentrations of BR and SA showed similar effects on plant fresh weight. No significant interaction effect was observed between salinity and BR or SA (Table 5). Results showed that salinity harms the plant dry weight of pepper (Table 4). BR and SA significantly improved plant dry weight. Plant dry weight ranged from 52.71 to 62.68 cm under BR treatments and from 53.29 to 56.87 cm under SA treatments. No significant interaction effect was observed between salinity and BR on plant fresh weight whereas significant interaction was observed between salinity and SA on plant dry weight (Table 5).

3.5. Effect of salicylic acid and brassinosteroid on root fresh and dry weight

Results showed that salinity has high significant negative effect on the root fresh weight (Table 6). BR and SA showed a significant positive effect on root fresh weight. Root fresh weight ranged between 34.64–37.90 g under BR treatments and between 37.67–50.56 g under SA treatments. No significant interaction effect was observed between salinity and BR or SA on root fresh weight (Table 7).

Results showed that salinity has high significant negative effect on the root dry weight (Table 6). BR and SA showed a significant positive effect on root fresh weight. Using BR and SA at 0.05 or 0.5 mM has a similar positive effect on root dry weight. Root dry weight ranged between 6.53–6.97 g under BR treatments and between 6.04–8.57 g under SA

Table 4
Effect of NaCl treatments, brassionosteroid (BR) and salicylic acid (SA) on plant fresh weight (g), plant dry weight (g) and SPAD parameters of sweet pepper

Main factor		Plant fresh weight (g)		Plant dry weight (g)		SPAD	
		BR	SA	BR	SA	BR	SA
NaCl treatments	Control	588.89^{a}	592.04^{a}	103.30^{a}	98.64^{a}	59.97^{a}	59.58^{a}
	50 mM	228.52^{b}	217.99^{b}	32.26^{b}	31.08^{b}	54.53^{a}	54.54^{a}
	150 mM	68.27^{c}	102.57^{b}	11.37^{b}	12.49^{b}	44.90^{b}	41.09^{b}
	p-value	***	***	***	***	***	***
Hormone treatment	Control	198.51^{b}	217.48^{b}	31.53^{b}	32.06^{a}	42.07^{b}	42.07^{b}
	0.05 mM	317.74^{ab}	365.53^{a}	52.71^{ab}	56.87^{a}	58.80^{a}	54.48^{a}
	0.5 mM	369.42^{a}	329.59^{ab}	62.68^{a}	53.29^{a}	58.53^{a}	58.67^{a}
	p-value	**	*	*	*	***	***

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

Table 5
Interaction effects of NaCl treatments, brassionosteroid (BR) and salicylic acid (SA) on plant fresh weight (g), plant dry weight (g) and SPAD parameters of sweet pepper

NaCl × Hormone	Plant fresh weight (g)		Plant dry weight (g)		SPAD	
	BR	SA	BR	SA	BR	SA
Control × 0 mM	460.70	460.70	72.50	72.50^{abc}	52.10	52.10^{bcd}
Control × 0.05 mM	541.97	671.03	99.10	110.60^{ab}	66.10	59.07^{abc}
Control × 0.5 mM	764.00	644.40	138.30	112.83^{a}	61.70	67.57^{a}
50 mM × 0 mM	95.87	95.87	11.83	11.83^{c}	38.80	38.80^{de}
50 mM × 0.05 mM	321.97	346.07	47.80	49.70^{bc}	63.60	64.87^{ab}
50 mM × 0.5 mM	267.73	212.03	37.13	31.70^{c}	61.20	59.97^{abc}
150 mM × 0 mM	38.97	95.87	10.27	11.83^{c}	35.30	35.30^{e}
150 mM × 0.05 mM	89.30	79.50	11.23	10.30^{c}	47.70	39.50^{de}
150 mM × 0.5 mM	76.53	132.33	12.60	15.33^{c}	52.70	48.47^{cde}
p-value	NS	NS	NS	**	NS	**

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

Table 6
Effect of NaCl treatments, brassionosteroid (BR) and salicylic acid (SA) on root fresh weight (g), root dry weight (g) and leaf area (cm) parameters of sweet pepper

Main factor		Root fresh weight (g)		Root dry weight (g)		Root/Shoot ratio	
		BR	SA	BR	SA	BR	SA
NaCl treatments	Control	57.02^{a}	60.34^{a}	11.33^{a}	11.21^{a}	0.11^{b}	0.11^{b}
	50 mM	22.96^{b}	29.26^{b}	3.47^{b}	4.26^{b}	0.12^{ab}	0.14^{ab}
	150 mM	15.31^{b}	16.51^{b}	1.69^{b}	2.03^{b}	0.15^{a}	0.16^{a}
	p-value	***	***	***	***	*	**
Hormone treatment	Control	19.74^{b}	17.89^{b}	2.97^{b}	2.89^{b}	0.12^{a}	0.11^{b}
	0.05 mM	34.64^{a}	37.67^{ab}	6.97^{a}	6.04^{ab}	0.13^{a}	0.12^{b}
	0.5 mM	37.90^{a}	50.56^{a}	6.53^{a}	8.57^{a}	0.12^{a}	0.18^{a}
	p-value	*	**	**	*	NS	***

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

Table 7
Interaction effects of NaCl treatments, brassionosteroid (BR) and salicylic acid (SA) on root fresh weight (g), root dry weight (g) and leaf area (cm) parameters of sweet pepper

NaCl × Hormone	Root fresh weight (g)		Root dry weight (g)		Root/Shoot ratio	
	BR	SA	BR	SA	BR	SA
Control × 0 mM	33.60	33.60^{b}	5.80	5.80^{b}	0.08	0.08
Control × 0.05 mM	70.30	53.77^{ab}	14.70	10.07^{ab}	0.15	0.09
Control × 0.5 mM	67.17	93.67^{a}	13.50	17.77^{a}	0.10	0.15
50 mM × 0 mM	10.03	10.03^{b}	1.43	1.43^{b}	0.13	0.13
50 mM × 0.05 mM	29.57	47.30^{ab}	4.70	6.73^{ab}	0.10	0.13
50 mM × 0.5 mM	29.27	30.43^{b}	4.27	4.60^{b}	0.12	0.15
150 mM × 0 mM	15.60	10.03^{b}	1.67	1.43^{b}	0.16	0.13
150 mM × 0.05 mM	13.07	11.93^{b}	1.67	1.33^{b}	0.14	0.13
150 mM × 0.5 mM	17.27	27.57^{b}	1.83	3.33^{b}	0.15	0.23
p-value	NS	*	NS	NS	NS	NS

*; $p < 0.05$, **; $p < 0.001$, ***; $p < 0.0001$ and NS; not significant

treatments. No significant interaction effect was observed between salinity and BR or SA on root dry weight (Table 7).

3.6. Effect of salicylic acid and brassinosteroid on root/shoot ratio

To test the effect of different NaCl levels on plant growth, the shoot/root ratio was calculated (Table 6). Obtained results show that NaCl significantly increased the root/shoot ratio. No significant effect of BR on root/shoot ratio was observed whereas SA at 0.5 mM significantly increased root/shoot ratio. No significant interaction effect was observed between salinity and BR or SA on root/shoot ratio (Table 7).

4. Discussion

Salinity is classified as one of the limiting factors in agriculture production, especially in arid and semiarid regions due to high evaporation, low precipitation rates and intensive agriculture system that usually associated with natural resources degradation, salinity reduces the growth and yield of crops through osmotic stress and toxic ions. This study reported the effect of salicylic acid and brassinosteroid on the performance of pepper (*Capsicum annum* L) under different salinity levels. Pepper is an important agricultural crop, not only because it is economically important, but also because of its nutritional value [13].

The analysis of yield components in this study revealed higher response for fruit number, fruit weight, plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll content and root/shoot ration. High NaCl concentration is toxic to plant growth, fruit set and eventually reduces yield quantity and quality [14].

The positive effect of SA and BR on the number of fruits could be due to their positive effect on plant metabolism and photosynthesis products leading to increase yield [15].

Sweet pepper plant fruit weight under salinity conditions revealed a decrease in weight as salinity levels increase. However, BR treatment and SA treatment reduce the effect of salinity and increase the fruit weight of the pepper plant. The positive effect on the fruit weight of the pepper plant could be due to SA and BR induced cell division and enlargement and increasing height of plant and chlorophyll content on leaves thus increase

photosynthesis and increase material that synthesis in the plant such as carbohydrates and proteins [16].

In the present study, plant height was reduced by NaCl. These results are in agreement with the results reported in chickpea [17,18] and pea [19]. The positive effect of SA on plant height could be because SA plays role in preserving auxin and cytokinin in the plant tissue that plays important role in cell division and elongation, and the BRs regulate both cell elongation and cell division and are essential for morphogenesis in darkness [5].

BR treatment and SA treatment reduce the effect of salinity on chlorophyll content. This is because SA has an important role in porphyrins synthesis that enter to building chlorophyll pigment molecule and increase protein and amino acid synthesis that leads to increase plastids division and increase chlorophyll pigments. Dubey [20] reported that the BR's improved chlorophyll content and stimulated the photosynthetic rate. Swamy and Rao [21] reported that external application of BR improved the photosynthesis rate and chlorophyll content in the geranium plant. BR and SA combination was effective in increasing the level of pigment in the maize leaves under NaCl stress [22]. The growth parameter including fresh and dry mass root part and shoot part decreased gradually with the rise of NaCl levels. These results agree with the results of Ghoulam et al. [22] who show that salinity caused a significant reduction in the growth of sugar beet plants.

The plants exposed to NaCl treatments and then treated with SA and BR show higher shoot fresh weight, root fresh weight, shoot dry weight and root dry weight compared to other treatments, this indicates that SA and BR application in low concentrations (0.05 or 0.5 mM) exhibited an increase in salt tolerance and effectively improved the growth of the sweet pepper plant under salinity stress. Similar results were reported in barley [23], cucumber [24], muskmelon [25] and basil [26]. Both root and shoot growth was affected by salt stress. The addition of salinity to the rooting medium inhibited the growth of the pepper plants' roots, but their growth rate was less affected by salts than the shoots, thus, root shoot ratio was increased with the higher NaCl level, however, without a significant effect [4] reported that shoot NaCl inhibits both cell division and cell enlargement.

5. Conclusion

The pepper plant is highly affected by the salinity in soil or irrigation water, application of hormones like salicylic acid and brassinosteroid revealed a positive effect on the stressed plant to reduce the negative effect of salinity. Salicylic acid and brassinosteroid have a significant effect in limiting salinity stress in growth parameters (fruit number, fruit weight, stem height, chlorophyll content etc.). In general, salicylic acid and brassinosteroid have a positive effect in reducing the negative effect of soil salinity on sweet pepper, especially at 50 mM NaCl.

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