MDPI

Article

Foliarly Applied 24-Epibrassinolide Modulates the Electrical Conductivity of the Saturated Rhizospheric Soil Extracts of Soybean under Salinity Stress

Victoria Otie [1,2], Ali Ibrahim [1], Itohowo Udo [1], Junichi Kashiwagi [3], Asana Matsuura [4], Yang Shao [5], Michael Itam [6], Ping An [2,*] and Anthony Egrinya Eneji [1]

1 Department of Soil Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, P.M.B. 1115, Calabar 540271, Nigeria
2 Arid Land Research Centre, Tottori University, Hamasaka, Tottori 6800001, Japan
3 Research Faculty of Agriculture, Hokkaido University, Kitaku, Kita9 Nishi9, Sapporo 0608589, Japan
4 Faculty of Agriculture, Shinshu University, 8304, Minamiminowa-Village, Kamiina-County, Nagano 3994598, Japan
5 College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China
6 Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
* Correspondence: an.ping@tottori-u.ac.jp

Abstract: The accumulation of salts within the rhizosphere is a common phenomenon in arid and semi-arid regions where irrigation water is high in salts. A previous study established the ameliorative effect of foliarly applied 24-epibrassinolide (BR) on soybean under salinity stress. As a follow-up to that study, this work evaluated the effects of BR on the electrical conductivity of saturated soil extracts (EC_{se}s) under soybean exposed to salt stress. Three salinity levels (3.24, 6.06 and 8.63 dS/m) in a factorial combination with six frequencies of BR application—control, seedling, flowering, podding, seedling + flowering and seedling + flowering + podding—were the treatments, and the rhizospheric EC_{se} was monitored from 3 to 10 weeks after the commencement of irrigation with saline water (WAST). The principal component analysis revealed that samples in saline BR treatments clustered together based on the BR application frequencies. There was a significant increase in EC_{se} with increases in salinity and WAST. The frequent application of BR significantly reduced EC_{se} to 5.07 and 4.83 dS/m relative to the control with 6.91 dS/m, respectively, at week 10. At 8.63 dS/m, the application of BR (seedling + flowering + podding) reduced EC_{se} by 31.96% compared with the control. The underlining mechanism is a subject for further investigation.

Keywords: arid region; soybean; 24-epibrassinolide; root exudates; rhizosphere; salinity stress

1. Introduction

Soil salinization is one of the major abiotic stresses encountered in agricultural environments. Its management can be achieved through the leaching and planting of salt-tolerant crops. Crop production in saline soils is inhibited by specific ions, as well as osmotic and toxic effects. Managing saline soil requires sustainable practices that maintain and improve the physicochemical properties of the soil, while attaining optimum production [1]. The major source of water for crop production in drylands is rainfall, which is often limited and irregular, and results in short-term crop productivity rather than long-term soil resource sustainability [2]. In semi-arid and arid regions, it is important to continuously monitor the electrical conductivity of the saturated soil extract (EC_{se}) in the rhizosphere to aid in the management of soil salinization for better crop production. It was previously reported that some drylands had high salt concentrations that rendered them inappropriate for agricultural purposes [3].

It was speculated that the world's population may increase from 6 to approximately 10 billion by 2050, and to meet the food demand of this growing populace, a larger increase

in food production is inevitable [4]. The advantages of amending sandy saline soils with organic inputs are generally short-lived due to its rapid decomposition under intense temperature and aeration, thereby causing a frequent yearly application to maintain soil productivity. Furthermore, improving sandy saline soils with inorganic fertilizers could often be unaffordable to peasant farmers [1,5]. A more stable compound such as 24-epibrassinolide (BR) could be used as an alternative to mitigate the deleterious effects of salinity on plant growth. Although considered a plant hormone that could regulate an array of physiological and developmental processes in plants under abiotic soil stressors [6–10], it was also found to modulate the biosynthesis of plants' primary metabolites, such as photosynthetic pigments, monosaccharides and protein, which are secreted as exudates through plant roots to the rhizosphere [11]. Exudates secreted from plant organs, including the roots, are involved in diverse interactions within the rhizospheric soil, such as chelating toxic compounds from the soil and changing the soil pH to help plants cope under stressed soil conditions [12].

In our previous report [10], we showed that BR caused significant increases in dry matter accumulation, protein content, photosynthetic activities, nutrient uptake and partitioning, as well as other physiological attributes and seed yield of soybean under salinity stress. However, there is a paucity of information on the influence of BR-treated plants on the rhizospheric electrical conductivity of the saturated soil extract (EC_{se}). Here, we hypothesized that BR could mediate the composition of root exudates to the advantage of plants growing in such stressed soils. This study aimed at evaluating the effects of the foliar application of BR at different growth stages of soybean, under varying salt concentrations on the rhizosphere EC_{se}.

2. Materials and Methods

2.1. Site Trial Management

This work is part of a previously published work [10]. Briefly, the experiment was conducted in a glasshouse of a subdivision of plant eco-physiology at the Arid Land Research Center (ALRC), Tottori University, Japan. The top soil (0–15 cm) used was analyzed in the previous study and the results are presented in Section 3.1 of this paper (Table 1). The treatments studied were three levels of salinity (3.24, 6.06 and 8.63 dS/m) in combination with six frequencies of 24-epibrassinolide (BR) applications {no application (control) and applications at seedling (when the trifoliolate leaves unfolded), flowering (when one flower opened at any node on the main stem), podding (when pods began to emerge on one of the four uppermost nodes on the main stem), seedling + flowering and seedling + flowering + podding stages) and eight monitoring durations of EC_{se} (3, 4, 5, 6, 7, 8, 9 and 10 weeks after the commencement of irrigation with saline water (WAST), which was the gestation period of the test crop—12 weeks)}. Treatments were laid out as a $3 \times 6 \times 8$ factorial, arranged in a completely randomized design (CRD) and replicated five times.

Soybean (cv. Tachiyutaka8428h) seeds were obtained from Denki nojo, Yamagata, Japan, while the BR was obtained from XenanXinyu Chemical Technology Company Limited (Ltd), Henan Province, China. The NaCl was procured from Gourmet Meat World Company Ltd., Tottori city, Japan. The three salinity levels (SLs = 3.24, 6.06 and 8.63 dS/m) were obtained by dissolving 1.75, 3.51 and 5.26 g of salt (NaCl), respectively, in 1 liter (L) of distilled water, and the corresponding concentration read from a hand-held CM-20E, TOA electrical conductivity meter (Tokyo TOA Electronics Limited (Ltd), Japan). Calibrated plastic pots with a height of 30 cm and diameter 25 cm, perforated at the bottom, were filled with 20 kg of sanddune soil and labelled according to treatment combinations. Soybean seeds were surface sterilized in 5 % sodium hypochlorite solution for 10 min and rinsed thoroughly in three changes of distilled water before sowing. Three seeds were sown per pot and later thinned to one vigorous plant at 10 days after sowing (DAS). Liquid fertilizer solution (NPK HYPONEX (6:10:5) produced by Kabushiki Corporation, Tokyo, Japan, was diluted at the rate of 5 mL in 1000 mL distilled water and applied as a blanket treatment on all the plants at 200 mL per pot, 2 and 5 weeks after sowing (WAS).

Table 1. Physicochemical properties of sanddune soils.

Soil Properties	Values
Sand (g/kg)	961.00
Silt (g/kg)	40.00
Clay (g/kg)	35.00
CEC (meq/100 g)	2.00
pH (H_2O)	5.98
EC_{se} (dS/m)	0.11
C (g/kg)	0.30
P (g/kg)	0.65
TN (g/kg)	0.10
Exchangeable Cation Content (meq/100 g)	
NH_4^+	0.00
K^+	0.06
Ca^{2+}	0.35
Mg^{2+}	0.68

TN—total nitrogen; EC_{se}—electrical conductivity of saturated soil extract.

The saline treatment started at 3 WAS by irrigating plants with saline water (500 mL/pot) based on the treatment specification each day until the end of the experiment. The BR solution was prepared by dissolving 1 g of 24-epibrassinolide powder in 20 L of distilled water and foliar sprayed at the rate of 200 mL per plant according to the manufacturer's recommendation. The application of BR was performed at 20 days after sowing (DAS) (seedling stage), 30 DAS (flowering stage) and 40 DAS (podding stage) using a calibrated hand sprayer.

2.2. Data Collection

The electrical conductivity of the saturated rhizospheric soil extract (EC_{se}) was determined starting from the first day of salinity treatment, and thereafter on a weekly basis till the tenth week after salinity treatment with the use of an electrical conductivity meter (CM-20E, TOA, Tokyo TOA Electronics Ltd., Kobe, Japan).

2.3. Statistical Analysis

To assess the interrelated effects of BR, SL and WAST on the electrical conductivity of the saturated soil extracts, a principal component analysis (PCA) was conducted using the FactoMineR package in R [13]. The data were further subjected to an analysis of variance using GenStat software edition 15.1 to partition the effects of the three factors and their interactions. Treatment means were compared using Duncan's new multiple range test at the 5% level of probability.

3. Results

3.1. Soil Analysis

Table 1 presents the results of the analysis of the physicochemical properties of the experimental soil. The soil was dominated by sand, followed by silt and clay. It was classified as *Entisols*, which are weather-resistant and can withstand extreme wetness or dryness. The soil was moderately acidic in the reaction, low in organic carbon, available phosphorus, total nitrogen, exchangeable cations (NH_4^+, K^+, Ca^{2+} and Mg^{2+}) and electrical conductivity of the saturated soil extract (EC_{se}). The soil also had a low (2.00 meq/100 g) cation exchange capacity, indicating poor inherent fertility.

3.2. Principal Component Analysis

The principal component analysis (PCA) revealed differences in the electrical conductivity of the saturated soil extracts (EC_{se}) obtained from different treatments. The principal components one (Dim 1) and two (Dim 2) explained 72.8% and 10.0%, respectively, of the variation in the EC_{se}. Dim 1 separated soil extracts based on salinity levels (SLs), while

Dim 2 separated them based on BR application frequencies (Figure 1a). The soil extracts without salt stress (control = SL_0) clustered together irrespective of BR application, whereas the salt-stressed (SL_n) samples clustered together based on the BR application frequencies, indicating the effectiveness of BR on the salt-treated extracts. Most of the treatment combinations had a similar contribution to the overall variation in the soil samples (Figure 1a). The soil extracts were also separated based on weeks after the commencement of irrigation with saline water (WAST) (Figure 1b). The samples from weeks 3 to 10 were separated on Dim 1. Most replicates from the same time point clustered together on the PCA, indicating sampling accuracy.

3.2.1. Effects of Salinity Levels, 24-Epibrassinolide and Weeks after the Commencement of Irrigation with Saline Water (WAST) on the Electrical Conductivity of the Saturated Soil Extracts in the Rhizosphere (EC_{se})

The main effects of salinity levels (SLs), 24-epibrassinolide (BR) and weeks after the commencement of irrigation with saline water (WAST) on the electrical conductivity of the saturated soil extracts in the rhizosphere (EC_{se}) are shown in Table 2. An increase in the salt concentration caused a significant ($p \leq 0.05$) increase in EC_{se}. The application of BR, irrespective of the growth stage, significantly ($p \leq 0.05$) reduced EC_{se} relative to no application (BR_0). The application of BR at BR_1, BR_2, BR_3, BR_4 and BR_5 reduced EC_{se} by 10.04%, 10.66%, 11.07%, 18.03% and 21.72%, respectively, compared with the control. However, frequent BR applications (BR_5—application at seedling + flowering + podding stages) caused the most significantly lowest salt concentrations in the rhizosphere. A successive increase in the weeks after irrigation with saline water led to a corresponding increase ($p \leq 0.05$) in the EC_{se} of the rhizospheric soil.
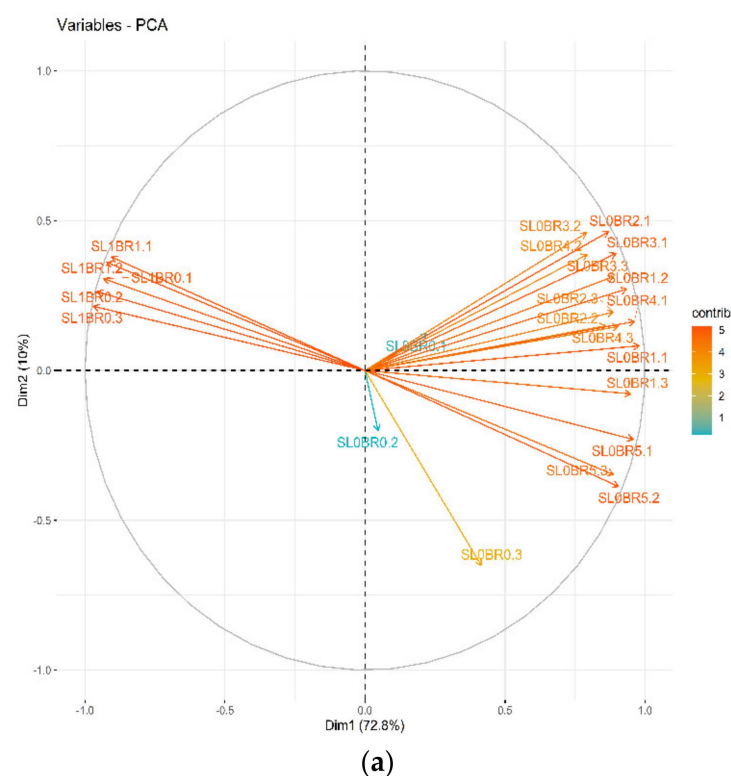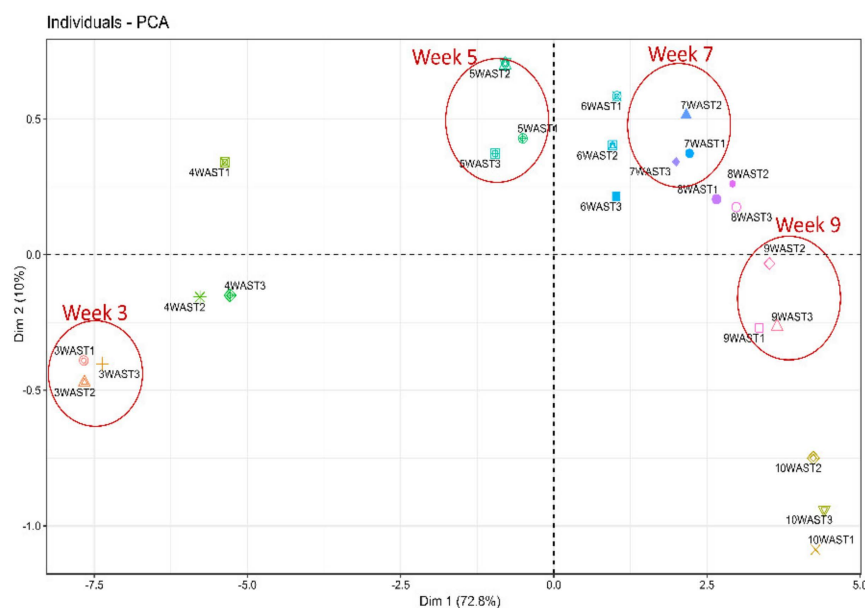

(a)

Figure 1. *Cont.*

(**b**)

Figure 1. (**a**) Principal component analysis of the effects of salinity levels and 24-epibrassinolide on EC_{se}; (**b**) principal component analysis on separation of soil extracts based on weeks after commencement of irrigation with saline water (WAST). SL0—0.11 dS/m; SL1—3.24 dS/m; SL2—6.06 dS/m; SL3—8.63 dS/m; BR—growth stages of 24-epibrassinolide application; BR0—control (no BR application); BR1—BR application seedling stage; BR2—BR application at flowering stage; BR3—BR application at podding stage; BR4—BR application at seedling + flowering stages; BR5—BR application at seedling + flowering + podding stages; EC_{se}—electrical conductivity of saturated soil extracts.

Table 2. Main effects of salinity levels (SLs), growth stages of application of 24-epibrassinolide (BR) and weeks after commencement of irrigation with saline water (WAST) on electrical conductivity of saturated soil extracts (EC_{se}s, dS/m) in soybean rhizosphere.

SL (dS/m)	EC_{se} (dS/m)	BR Application Stages	EC_{se} (dS/m)	WAST	EC_{se} (dS/m)
3.24	3.37_c	No Application	4.88_a	3	1.91_h
6.06	4.38_b	Seedling	4.39_b	4	2.57_g
8.63	5.15_a	Flowering	4.36_b	5	4.04_f
		Podding	4.34_b	6	4.61_e
		Seedling + Flowering	4.00_c	7	4.97_d
		Seedling + Flowering + Podding	3.82_d	8	5.20_c
				9	5.42_b
				10	5.68_a

For each main effect, mean pairs with different letters were significantly different at the 5% probability level according to Duncan's new multiple range test.

3.2.2. Interaction Effects of Growth Stages of Application of BR and Salinity Concentrations on Electrical Conductivity of the Saturated Soil Extracts (EC_{se}s) in Rhizospheric Soil

Table 3 shows the interaction effects of the growth stages of the application of BR and salinity concentrations on the electrical conductivity of the saturated soil extract (EC_{se}s) in the rhizospheric soil of soybean. At each stage of BR application, including the BR0 (control), increases in salinity levels (SLs) significantly increased the EC_{se}. At all SLs, the BR application reduced ($p \leq 0.05$) the rhizospheric EC_{se} relative to no application (BR0). However, at each SL, a one-time application of BR (i.e., BR1—seedling stage; BR2—flowering stage; BR3—podding stage) had a statistically similar rhizospheric EC_{se}. The application of BR at BR4, the seedling + flowering stage, or BR5, reduced ($p \leq 0.05$) the rhizospheric soil

EC_{se} compared with other stages of application. At the highest concentration of salinity (8.63 dS/m), the application of BR at BR_1, BR_2 BR_3, BR_4 and BR_5 reduced EC_{se} by 12.46%, 11.95%, 12.46%, 19.87% and 23.23%, respectively, relative to the control.

Table 3. Interaction effects of growth stages of 24-epibrassinolide application (BR) and salinity levels (SLs) on electrical conductivity of saturated soil extracts (EC_{se}s, dS/m) in soybean rhizosphere.

BR Application Stages	Salinity Levels (SLs)		
	SL_1 (dS/m)	SL_2 (dS/m)	SL_3 (dS/m)
No Application	3.80_i	4.91_c	5.94_a
Seedling	3.49_j	4.49_{ef}	5.20_b
Flowering	3.47_j	4.39_{fg}	5.23_b
Podding	3.48_j	4.34_g	5.20_b
Seedling + Flowering	3.12_k	4.13_h	4.76_d
Seedling + Flowering + Podding	2.89_l	4.01_h	4.56_e

Mean pairs with different letters were significantly different at the 5% probability level according to Duncan's new multiple range test. SL_1—3.24 dS/m; SL_2—6.06 dS/m; SL_3—8.63 dS/m.

3.2.3. Interaction Effects of Growth Stages of BR Application and Weeks after Commencement of Irrigation with Saline Water (WAST) on EC_{se}

The interaction effects of the growth stages of BR application and weeks after the commencement of irrigation with saline water (WAST) on EC_{se} are shown in Table 4. There was a significant increase in rhizospheric EC_{se} with successive increases in WAST with no BR applied (BR_0) and when applied only once at BR_1, BR_2 and BR_3. However, when BR was applied twice (BR_4) or thrice (BR_5), the rhizospheric EC_{se} was statistically similar from week 7 to week 10. At weeks 4, 5 and 6 after the commencement of saline water irrigation, the BR_1, BR_2, BR_3 and BR_4 treatments had statistically similar rhizospheric EC_{se}s. However, from week 7 to week 10, BR_4 and BR_5 significantly reduced the rhizospheric EC_{se} relative to other stages of application. At week 10, the BR application at BR_1, BR_2, BR_3, BR_4 and BR_5 reduced EC_{se} by 16.21%, 16.93%, 16.93%, 26.63% and 23.23%, respectively, relative to no application.

Table 4. Interaction effects of growth stages of 24-epibrassinolide application (BR) and weeks after commencement of irrigation with saline water (WAST) on electrical conductivity of the saturated soil extracts (EC_{se}s) in soybean rhizosphere.

BR Application Stages	Weeks after Commencement of Irrigation with Saline Water							
	3	4	5	6	7	8	9	10
No Application	2.62_r	3.11_q	4.47_{lm}	4.95_{fghi}	5.30_e	5.63_{cd}	6.07_b	6.91_a
Seedling	1.88_t	2.61_r	4.14_{no}	4.70_{j-l}	5.01_{fgh}	5.41_{de}	5.58_{cd}	5.79_c
Flowering	2.02_t	2.53_{rs}	4.04_{no}	4.65_{j-l}	5.05_{fg}	5.32_e	5.56_{cd}	5.74_c
Podding	1.86_t	2.50_{rs}	4.01_o	4.64_{j-l}	5.08_f	5.31_e	5.56_{cd}	5.74_c
Seedling + Flowering	1.56_u	2.37_s	3.94_o	4.49_{lm}	4.77_{h-k}	4.86_{f-j}	4.96_{fghi}	5.07_{fg}
Seedling + Flowering + Podding	1.50_u	2.30_s	3.64_p	4.26_{mn}	4.58_{kl}	4.65_{j-l}	4.76_{i-k}	4.83_{g-j}

Mean pairs with different letters are significantly different at the 5% probability level according to Duncan's new multiple range test.

3.2.4. Interaction Effects of Salinity Levels and Weeks after Commencement of Irrigation with Saline Water

Table 5 shows the interaction effects of salinity levels (SLs) and weeks after the commencement of irrigation with saline water on the electrical conductivity of the saturated soil extracts. For each salinity concentration, successive increases in weeks after saline water irrigation led to corresponding significant increases in rhizospheric EC_{se}. The same trend was observed at each week with successive increases in salinity.

Table 5. Interaction effects of salinity levels (SLs) and weeks after commencement of irrigation with saline water (WAST) on electrical conductivity of the saturated soil extracts (EC_{se}s, dS/m) in soybean rhizosphere.

SL (dS/m)	Weeks after the Commencement of Irrigation with Saline Water							
	3	4	5	6	7	8	9	10
3.24	1.29_S	1.80_q	3.18_n	3.67_m	3.97_l	4.19_k	4.35_j	4.55_i
6.06	1.63_r	2.59_p	3.84_l	4.87_h	5.12_g	5.34_f	5.67_e	5.96_{cd}
8.63	2.81_o	3.32_n	5.11_g	5.30_f	5.81_{de}	6.06_c	6.23_b	6.52_a

Mean pairs with different letters were significantly different at the 5% probability level according to Duncan's new multiple range test.

3.2.5. Three-Way Interaction Effects of BR Application Stages, Salinity Levels and Weeks after Commencement of Irrigation with Saline Water on the Electrical Conductivity of the Rhizospheric Saturated Soil Extracts

As Table 6 shows, although the interactions among the factors were not significant ($p > 0.05$), with or without BR application, there was an increase in EC_{se} from week 3 to week 10, irrespective of salinity level. The highest EC_{se} was observed in the rhizosphere with no BR application and irrigated with saline water (SL_3 = 8.63 dS/m) at the tenth week (week 10). There was a great decrease in EC_{se} with frequent applications of BR (BR_4 and BR_5) at each respective salinity level and weeks after saline water irrigation, relative to other growth stages of BR application. At week 10 after the commencement of irrigation with saline water, and at the highest salinity concentration of 8.63 dS/m, BR application at BR_1, BR_2, BR_3, BR_4 and BR_5 reduced EC_{se} by 20.53%, 20.78%, 21.14%, 30.01% and 31.96%, respectively, compared with the control.

Table 6. Interaction effects of growth stages of application of 24-epibrassinolide (BR), salinity levels (SLs) and weeks after commencement of irrigation with saline water (WAST) on electrical conductivity of the saturated soil extracts (EC_{se}s) in rhizosphere of soybean.

BR Application Stages	SL (dS/m)	Weeks after Commencement of Irrigation with Saline Water							
		3	4	5	6	7	8	9	10
No application	3.24	1.83_a	2.30_a	3.32_a	3.87_a	4.28_a	4.65_a	4.94_a	5.24_a
	6.06	2.19_a	3.03_a	4.53_a	5.05_a	5.29_a	5.75_a	6.15_a	7.27_a
	8.63	3.85_a	4.01_a	5.56_a	5.93_a	6.33_a	6.49_a	7.11_a	8.23_a
Seedling	3.24	1.32_a	1.67_a	3.26_a	3.79_a	4.08_a	4.37_a	4.57_a	4.85_a
	6.06	1.71_a	2.76_a	3.99_a	4.97_a	5.11_a	5.59_a	5.78_a	5.97_a
	8.63	2.60_a	3.40_a	5.18_a	5.33_a	5.84_a	6.29_a	6.39_a	6.54_a
Flowering	3.24	1.38_a	1.60_a	3.21_a	3.74_a	4.08_a	4.37_a	4.55_a	4.81_a
	6.06	1.70_a	2.67_a	3.77_a	4.94_a	5.13_a	5.29_a	5.72_a	5.89_a
	8.63	2.97_a	3.32_a	5.16_a	5.25_a	5.93_a	6.29_a	6.41_a	6.52_a
Podding	3.24	1.30_a	1.89_a	3.15_a	3.70_a	4.04_a	4.35_a	4.57_a	4.81_a
	6.06	1.64_a	2.41_a	3.71_a	4.94_a	5.14_a	5.25_a	5.74_a	5.92_a
	8.63	2.62_a	3.21_a	5.18_a	5.27_a	6.07_a	6.33_a	6.38_a	6.49_a
Seedling + Flowering	3.24	0.99_a	1.69_a	3.11_a	3.56_a	3.83_a	3.88_a	3.91_a	4.00_a
	6.06	1.18_a	2.38_a	3.56_a	4.79_a	5.11_a	5.19_a	5.38_a	5.44_a
	8.63	2.52_a	3.05_a	5.16_a	5.10_a	5.38_a	5.52_a	5.58_a	5.76_a
Seedling + Flowering + Podding	3.24	0.91_a	1.64_a	3.03_a	3.33_a	3.49_a	3.53_a	3.57_a	3.60_a
	6.06	1.33_a	2.30_a	3.49_a	4.52_a	4.92_a	4.99_a	5.23_a	5.30_a
	8.63	2.26_a	2.96_a	4.41_a	4.93_a	5.33_a	5.43_a	5.49_a	5.60_a

Mean pairs with different letters were significantly different at the 5% probability level according to Duncan's new multiple range test.

4. Discussion

The monitoring of the in situ electrical conductivity of saturated soil extracts (EC_{se}s) of rhizospheric soils is an important management tool aimed at determining the extent and severity of salinization processes. Overtime, the extent of the salinization of croplands

may increase, resulting in accelerated land degradation and desertification, decreased farm output and, consequently, jeopardizing the environment and food security, particularly in drylands [14]. Such soils lack distinct horizons, an optimal condition for agricultural soils and are generally low in organic matter/carbon, natural fertility and water-holding capacity [15].

Increases in salinity levels significantly elevated the EC_{se} in the soil rhizosphere of soybean plants and peaked at the highest concentration. This was possibly a result of excessive accumulations of Na^+ and Cl^- due to high water evaporation; such a concentration of salts could possibly induce ion toxicity [16] in soils. Excessive Na^+ in the soil would decrease the uptake of NH_4^+ and other base cations (K^+, Ca^{2+} and Mg^{2+}) from the soil's exchange complex. Although, Cl^- is not readily adsorbed, it does move with soil–water and is easily taken up by plants through enzymes in the root cell membrane, thereby reducing the absorption of nitrates from the soil [17]. It was estimated that by the year 2050, more than 50% of the arable land across the globe would have been salinized [18]. However, various agronomic practices, including exogenously applied growth regulators, can mitigate the adverse effects of different abiotic stresses such as drought, heavy metals as well as salt stress [10,19,20].

24-epibrassnolide (BR), an active by-product from brassinolide biosynthesis is one of the stress ameliorative approaches adopted to improve and stimulate different plant metabolic processes. It is known to improve the efficiency of transpiration and accelerates metabolic processes that favor the accumulation and release of carbohydrates in the form of gluconic acids, amino acids and organic acids [21] in plant organs (leaves, shoots or roots) as exudates to their surrounding media. These compounds are involved in numerous interactions within the rhizosphere, and could contribute to the decline in EC_{se} and salts, including Na^+ and Cl^- [22].

In this study, the separation of salt extract on the PCA based on BR applications demonstrated its effectiveness in reducing the EC_{se} of saline soils. It further proved to be sufficient in ameliorating the detrimental effects of salinity on soybean when applied at maximum frequencies of BR_4 and/or BR_5 growth stages. The sampling accuracy indicated by the clustering of similar data points on the PCA further confirmed these findings. The effects could have occurred through changes in salt concentrations in the rhizospheric zones due to exudates from plant roots. Exudates are primary and secondary metabolites (carbohydrates, amino acids, organic acids, flavonoids, glucosinolates, auxins, etc.) secreted by different plant organs, including roots, to their surrounding environment [23]. These metabolites have been identified and quantified in different plant species, including soybean or the common bean [24,25]. The exogenous application of BR was recently reported to boost the yield and dry matter (DM) production in soybean and rice under salinity or high-temperature stress [10,26]. The increased DM production could enhance the secretion of exudates from plant roots to complex with salt ions and significantly reduce the EC_{se} in the rhizospheric soil. Root exudates (flavonoids) obtained from soybean and the common bean whose concentrations increased when plants were under salt stress [27,28] could mitigate both osmotic and ion toxicity in the rhizosphere. Similarly, the excised roots of almond trees cultured under in vitro conditions exuded proline in larger quantities when under salt stress [29].

There was a substantial increase in EC_{se} across the weeks after the saline water (WAST) treatment. In saline drylands, where high evaporation rates tend to concentrate the water solution, natural salinization can occur regardless of anthropogenic activities, especially when cations and anions are easily leached down the aquifers, resulting in a relative increase in Na^+ ions, which, consequently, may replace Ca^{2+} and Mg^{2+} in the soil exchange complex [30]. Although plants take up many salts in the form of nutrients, when more salt is added to the soil than required, it poses a threat to plants. For the various salt concentrations used in this study, there was a gradual increase in EC_{se} with the number of weeks after the commencement of the salinity treatment, becoming extremely high at the peak of the duration (week 10). Such a high EC_{se} (as a result of excessive sodium

ions accumulated overtime) may reduce the soil's ability to conduct water and cause the accumulation of a salt crust on the soil's surface that could leach down during an unexpected precipitation to cause injury to plant roots [31].

The interaction between BR and salinity levels showed that an increase in BR application frequency significantly reduced the EC_{se} across various salinity levels. The exogenous application of BR was reported to reduce Na^+ and Cl^- contents, while increasing N, P, K and Ca to boost the activities of antioxidant enzymes, leading to improved plant yield under salinity stress [32]. The capacity of BR to play this mitigating role could possibly be attributed to its ability to induce the secretion of metabolites from different parts of the plant root system into the soil environment during growth [33]. In a recent study, the contents of primary metabolites, such as photosynthetic pigments, monosaccharides and protein, improved the growth rate of duckweed (*Wolffia arrhiza*) when sprayed with BR [34]. Root exudates serve as a material exchange and information transmission between plants and the soil in maintaining the functionality and vitality of the rhizosphere [35]. The root secretions could alter the rhizospheric soil environment, which is a major adaptive response mechanism for plants to cope with environmental abiotic stresses such as salinity [36].

The temporal effects of 24-epibrassinolide on the EC_{se} with the number of weeks after the commencement of irrigation with saline water confirmed the efficacy of BR in accelerating metabolic processes that enhanced the accumulation of soluble proteins, and the stability or synthesis of organic acids and monosaccharides, mainly glucose, during photosynthesis [37]. This was evident across the weeks of saline water irrigation, where it significantly reduced the EC_{se} to boost soybean yield across the weeks of measurement. The higher frequency of BR application may have promoted the synthesis of metabolites, thereby contributing to an increased plant biomass and, possibly, root exudates, as beneficial support to soybean growth under prolonged salinity stress.

Soils are complex entities that provide substrates for nature as well as agriculture. Saline soils inhibit the growth of most crops because of the higher concentration of neutral soluble salts in them. This occurs majorly through irrigation water, which is usually a gradual process where the salts accumulate over time before their effects are visible. Most salts are taken up by plants in the form of nutrients, but when the soil receives more salt than is removed, plants are eventually affected by salinity injuries [38]. The increase in salt concentration in our rhizospheric soil increased the EC_{se} as the saline irrigation week progressed. The plants showed some growth inhibitions from the SL_2 (6.06 dS/m) at week 7, whereas a greater growth inhibition occurred at week 5 when the salt concentration was highest at SL_3 (8.63 dS/m) as compared with SL_1 (3.24 dS/m) [10]. This trend could be attributed to the fact that the soil physicochemical properties and ecological balance were altered by excessive salt accumulations overtime, leading to high osmotic stress, nutritional disorders and toxicities in the plants [39]. Soybean is considered to have a moderately tolerant threshold of approximately 5.0 dS/m of saturated soil extract [40]. The highest frequency of BR application (BR_5) was able to reduce EC_{se} to 4.99 dS/m at week 9 and 4.93 dS/m at week 6 with saline irrigation water of 6.06 and 8.63 dS/m, respectively. This indicated that the plant was substantially protected at its vegetative and reproductive growth stages when considering the 5.0 dS/m EC_{se} tolerance threshold.

There was an improvement in the general morphological and physiological wellbeing of soybean plants when BR was exogenously applied. As reported previously [41], the continuous application of BR improved the biosynthesis of targeted compounds in the plants and facilitated the secretion of primary metabolites that suppressed the abiotic environment. The successive increase in salinity increased the EC_{se} across the weeks of saline treatment. The highest EC_{se} was under SL_3, without BR. The application of BR reduced the EC_{se} substantially between weeks 5 and 7 at SL_2 or SL_3 as compared with BR_0. However, repeat use of BR (BR_4 or BR_5) significantly reduced the EC_{se} up to week 9, whether at SL_2 or SL_3, relative to BR_1, BR_2 or BR_3. As previously reported [10], soybean responds to different salinity levels in terms of both the total biomass yield and yield variables. Generally, a lower yield was observed at higher salinity levels. The BR

aided the mobilization of nutrients through the exuded metabolites. Plants add glucose to rhizospheres through exudation to reduce electrolyte concentrations and facilitate their survival in saline drylands [42].

5. Conclusions

Our study concluded that 24-epibrassinolide, a phytohormone that regulates several physiological processes and increases plant contents of primary metabolite, could also confer tolerance to salinity stress by modulating the electrical conductivity of saturated soil extract (EC_{se}) in the rhizosphere of soybean plants, possibly through root exudates. The frequent application of BR (seedling + flowering + podding) may not only mitigate the injurious effects of salt on plants, but could also reduce the high EC_{se} in the rhizosphere due to saline water irrigation. Based on the findings of this work, the quality and quantity of root exudates of BR-treated plants exposed to salinity should be evaluated to further explain its role in modulating the EC_{se}.

Author Contributions: Conceptualization, V.O., A.I., I.U., P.A. and A.E.E.; methodology, V.O., I.U., A.I., P.A. and A.E.E.; software, V.O., Y.S., I.U., J.K. and M.I.; validation, V.O., I.U., A.I., A.M., M.I. and P.A.; formal analysis, I.U., M.I. and V.O.; investigation, V.O., I.U., M.I. and J.K.; resources, V.O., I.U., A.I. and P.A.; data curation, V.O., I.U. and M.I.; writing—original draft preparation, V.O.; writing—review and editing, I.U., A.I., M.I., P.A. and A.E.E.; visualization, A.I., Y.S., A.M., J.K. and P.A.; supervision, P.A. and A.E.E.; project administration, V.O. and P.A.; funding acquisition, P.A. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

References

1. Mando, A.; Ouattara, B.; Sedogo, M.; Stroosnijder, L.; Ouattara, K.; Brussaard, L.; Vanlauwe, B. Long-Term Effect of Tillage and Manure Application on Soil Organic Fractions and Crop Performance Under Sudano-Sahelian Conditions. *Soil Till. Res.* **2005**, *80*, 95–101. [CrossRef]
2. United Nation Population Division. *World Population Prospects (Sustaining Water)*; The United Nations: New York, NY, USA, 1994.
3. An, P.; Kajiwara, T.; Inoue, T.; Li, X.; Inanaga, S. Cultivation of Halophytes in Saline Soils: A Case Study of the Saline Soils Around Bohai Bay, China. *Sand Dune Res.* **2013**, *60*, 9–18.
4. United Nations. Department of Economic and Social Affairs, Population Division. In *World Population Prospects: Comprehensive Tables ST/ESA/SER.A/336*; United Nations: New York, NY, USA, 2012; Volume 1.
5. Topoliantz, S.; Ponge, J.F.; Ballof, S. Manioc Peel and Charcoal: A Potential Organic Amendment for Sustainable Soil Fertility in the Tropics. *Biol. Fert. Soils* **2005**, *41*, 15–21. [CrossRef]
6. Fariduddin, Q.; Ahmed, M.; Mir, B.A.; Yusuf, M.; Khan, T.A. 24-Epibrassinolide Mitigates the Adverse Effects of Manganese Induced Toxicity Through Improved Antioxidant System and Photosynthetic Attributes in *Brassica juncea*. *Environ. Sci. Pollut. Res.* **2015**, *22*, 11349–11359. [CrossRef]
7. Otie, V.; Ping, A.; Udo, I.; Eneji, E.A. Brassinolide Effects on Maize (*Zea Mays* L.) Growth and Yield Under Waterlogged Conditions. *J. Plant. Nutr.* **2019**, *42*, 954–969. [CrossRef]
8. Otie, V.; An, P.; Ali, I.; Eneji, E.A. Plant Growth Regulator-Brassinolide for Mitigating Field Waterlogging Stress on Maize. *Int. J. Plant Soil Sci.* **2019**, *30*, 1–14. [CrossRef]
9. Lu, J.; Zong, X.F.; Ahmad, A.S.; Wu, X.; Wu, C.; Li, Y.P.; Wang, S.G. Alteration in Morpho-Physiological Attributes of *Leymus Chinensis* (Trin.) Tzvelev By Exogenous Application of Brassinolide Under Varying Levels of Drought Stress. *Chil. J. Agric. Res.* **2020**, *80*, 61–71. [CrossRef]
10. Otie, V.; Udo, I.; Shao, Y.; Itam, M.O.; Okamoto, H.; An, P.; Eneji, E.A. Salinity Effects on Morpho-Physiological and Yield Traits of Soybean (*Glycine Max* L.) As Mediated by Foliar Spray with Brassinolide. *Plants* **2021**, *10*, 541. [CrossRef]

11. Bajguz, A. Effect of Brassinosteroids on Nucleic Acids and Protein Content in Cultured Cells of *Chlorella vulgaris*. *Plant Physiol. Biochem.* **2000**, *38*, 209–215. [CrossRef]

12. Jones, D.L.; Nguyen, C.; Finlay, R.D. Carbon Flow in the Rhizosphere: Carbon Trading at the Soil-Root Interface. *Plant Soil* **2009**, *321*, 5–33. [CrossRef]

13. Lê, S.; Josse, J.; Husson, F. FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Softw.* **2008**, *25*, 1–18. [CrossRef]

14. Cuevas, J.; Daliakopoulos, I.N.; del Moral, F.; Hueso, J.J.; Tsanis, I.K. A review of Soil-Improving Cropping Systems for Soil Salinization. *Agron.* **2019**, *9*, 295. [CrossRef]

15. Weil, R.R.; Brady, N.C. *The Nature and Properties of Soils*, 15th ed.; Pearson Education: Colunbus, OH, USA, 2016; pp. 421–438.

16. Litalien, A.; Zeeb, B. Curing the Earth: A review of Anthropogenic Soil Salinization and Plant-Based Strategies for Sustainable Mitigation. *Sci. Total Environ.* **2020**, *698*, 134235. [CrossRef] [PubMed]

17. Hasana, R.; Mayake, H. Salinity Stress Alters Nutrient Uptake and Causes the Damage of Root and Leaf Anatomy in Maize. *KnE Life Sci.* **2017**, *3*, 219–225. [CrossRef]

18. Jamil, A.; Riaz, S.; Ashraf, M.; Foolad, M.R. Gene Expression Profiling of Plants Under Salt Stress. *Crit. Rev. Plant Sci.* **2011**, *30*, 435–458. [CrossRef]

19. Anjum, S.A.; Tanveer, M.; Hussain, S.; Tung, S.A.; Samad, R.A.; Wang, L.; Khan, I.; Rehman, N.; Shah, A.N.; Shahzad, B. Exogenously Applied Methyl Jasmonate Improves the Drought Tolerance in Wheat Imposed at Early and Late Developmental Stages. *Acta Physiol. Plant.* **2016**, *38*, 25. [CrossRef]

20. Shahzad, B.; Tanveer, M.; Che, Z.; Rehman, A.; Cheema, S.A.; Sharma, A.; Song, H.; Rehman, S.; Zhaorong, D. Role of 24-epibrassinolide (EBL) in mediating heavy metal and pesticide induced oxidative stress in plants: A review. *Ecotoxicol. Environ. Safety* **2018**, *147*, 935–944. [CrossRef]

21. Hussain, M.A.; Fahad, S.; Sharif, R.; Jan, M.F.; Mujtaba, M.; Ali, Q.; Ahmad, A.; Ahmad, H.; Amin, N.; Ajayo, B.S.; et al. Multifunctional Role of Brassinosteroid and Its Analogues in Plants. *Plant Growth Regul.* **2020**, *92*, 141–156. [CrossRef]

22. Pandey, C.B.; Kumar, P.; Chaudhari, S.K. Root Exudates Reduce Electrical Conductivity and Water Potential of Rhizospheres and Facilitate Non-Halophytes to Survive in Dry Land Saline Soils. *Trop. Ecol.* **2017**, *58*, 705–716.

23. Sasse, J.; Martinoia, E.; Northen, T. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends Plant Sci.* **2018**, *23*, 25–41. [CrossRef]

24. Tawaraya, K.; Horie, R.; Saito, S.; Wagatsuma, T.; Saito, K.; Oikawa, A. Metabolite Profiling of Root Exudates of Common Bean Under Phosphorus Deficiency. *Metabolites* **2014**, *4*, 599–611. [CrossRef] [PubMed]

25. Tawaraya, K.; Horie, R.; Shinano, T.; Wagatsuma, T.; Saito, K.; Oikawa, A. Metabolite Profiling of Soybean Root Exudates Under Phosphorus Deficiency. *Soil Sci. Plant Nutr.* **2014**, *60*, 679–694. [CrossRef]

26. Chen, Y.H.; Chen, H.Z.; Xiang, J.; Zhang, Y.K.; Wang, Z.G.; Zhu, D.F.; Wang, J.K.; Zhang, Y.P.; Wang, Y.L. Rice Spikelet Formation Inhibition Caused by Decreased Sugar Utilization Under High Temperature Is Associated with Brassinolide Decomposition. *Environ. Exp. Bot.* **2021**, *190*, 104585. [CrossRef]

27. Dardanelli, M.S.; Manyani, H.; González-Barroso, S.; Rodríguez-Carvajal, M.A.; Gil-Serrano, A.M.; Espuny, M.R.; López-Baena, J.; Bellogín, R.A.; Megías, M.; Ollero, F.J. Effect of the Presence of the Plant Growth Promoting Rhizobacterium (PGPR) *Chryseobacterium Balustinum* Aur9 and Salt Stress in the Pattern of Flavonoids Exuded by Soybean Roots. *Plant Soil* **2010**, *328*, 483–493. [CrossRef]

28. Dardanelli, M.S.; de Córdoba, F.J.F.; Estévez, J.; Contreras, R.; Cubo, M.T.; Rodríguez-Carvajal, M.A.; Gil-Serrano, A.M.; López-Baena, F.J.; Bellogín, R.; Manayni, H.; et al. Changes in Flavonoids Secreted by *Phaseolus Vulgaris* Roots in The Presence of Salt and the Plant Growth-Promoting Rhizobacterium *Chryseobacterium balustinum*. *Appl. Soil Ecol.* **2012**, *57*, 31–38. [CrossRef]

29. Marin, J.A.; Andreu, P.; Carrasco, A.; Arbeloa, A. Determination of Proline Concentration, An Abiotic Stress Marker, In Root Exudates of Excised Root Cultures of Fruit Tree Rootstocks Under Salt Stress. *Rev. Régions Arid.* **2010**, *24*, 722–727.

30. Bui, E.N. Causes of Soil Salinization, Sodification, and Alkalinization. In *Oxford Research Encyclopedia of Environmental Science*; Oxford University Press: Oxford, UK, 2017.

31. Zhang, J.G.; Xu, X.W.; Li, S.Y. Danger of Sudden Strong Rainfall to Calligonum-Take the Tarim Desert Highway Shelterbelt Eco-Project as an Example. In *Association for Science and Technology of Xinjiang Uygur Autonomous Region. Collected Papers of the 6th Youth Academic Year and the 1st Doctoral Forum in Xinjiang*; Xinjiang Science and Technology Press: Urumqi, China, 2006; Volume 3, pp. 1261–1266. (In Chinese)

32. Kaya, C.; Aydemir, S.; Akram, N.A.; Ashraf, M. Epibrassinolide Application Regulates Some Key Physio-Biochemical Attributes as Well as Oxidative Defense System in Maize Plants Grown Under Saline Stress. *J. Plant Growth Regul.* **2018**, *37*, 1244–1257. [CrossRef]

33. Roh, J.; Yeom, H.S.; Jang, H.; Kim, S.; Youn, J.H.; Kim, S.K. Identification and Biosynthesis of C-24 Ethylidene Brassinosteroids in *Arabidopsis thaliana*. *J. Plant Biol.* **2017**, *60*, 533–538. [CrossRef]

34. Chmur, M.; Bajguz, A. Brassinolide Enhances the Level of Brassinosteroids, Protein, Pigments, and Monosaccharides in *Wolffia arrhiza* treated with brassinazole. *Plants* **2021**, *10*, 1311. [CrossRef]

35. Neumann, G. Root Exudates and Nutrient Cycling. In *Nutrient Cycling in Terrestrial Ecosystems*; Marschner, P., Rengel, Z., Eds.; Springer: Berlin/Heidelberg, Germany, 2007; pp. 123–157.

36. Zhao, M.; Zhao, J.; Yuan, J.; Hale, L.; Wen, T.; Huang, Q.; Vivanco, J.M.; Zhou, J.; Kowalchuk, G.A.; Shen, Q. Root Exudates Drive Soil-Microbe-Nutrient Feedbacks in Response to Plant Growth. *Plant Cell Environ.* **2021**, *44*, 613–628. [CrossRef]

37. Eckstein, A.; Zieba, P.; Gabrys, H. Sugar and Light Effects on the Condition of the Photosynthetic Apparatus of *Arabidopsis thaliana* Cultured In Vitro. *J. Plant Growth Regul.* **2012**, *31*, 90–101. [CrossRef]

38. Shrivastava, P.; Kumar, R. Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria as One of the Tools for Its Alleviation. *Saudi J. Biol. Sci.* **2015**, *22*, 123. [CrossRef]

39. Gill, S.S.; Tuteja, N. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef] [PubMed]

40. Bustingorri, C.; Lavado, R.S. Soybean Growth Under Stable Versus Peak Salinity. *Sci. Agric.* **2011**, *68*, 102. [CrossRef]

41. Yuan, J.; Zhao, J.; Wen, T.; Zhao, M.; Li, R.; Goossens, P.; Huang, Q.; Bai, Y.; Vivanco, J.M.; Kowalchuk, G.A.; et al. Root Exudates Drive the Soil-Borne Legacy of Aboveground Pathogen Infection. *Microbiome* **2018**, *6*, 156. [CrossRef] [PubMed]

42. Sacchi, G.A.; Abruzzese, A.; Lucchini, G.; Fiorani, F.; Cocucci, S. Efflux and Active Re-Adsorption of Glucose in Roots of Cotton Plants Grown Under Saline Conditions. *Plant Soil* **2000**, *220*, 1–11. [CrossRef]