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Biostimulant Effect and Antioxidant Responses of Carrot Extract and the Viability of Rice Seeds Under Salt Stress

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Abstract: Bioactive compounds in plants, such as carrots, have been widely used for their benefits. In agriculture, their potential as biostimulants still needs to be investigated, especially for their possible antioxidant action in plants subjected to abiotic stresses, such as salinity. This work aimed to evaluate the elicitor potential of carrot extract in alleviating salt stress in rice plants. This study aimed to evaluate the elicitor potential of carrot extract in alleviating saline stress in the rice cultivars BRS Querência and BRS 358. Aqueous extracts of carrot roots at concentrations of 0% (water), 25%, 50%, and 100% were used to soak rice seeds for 48 h, which were then subjected to different concentrations of NaCl (0, 25, 75, and 150 mM). To determine the effect of carrot extract as an elicitor under saline stress conditions, the following tests were conducted: germination, seedling length, dry mass, and oxidative stress through the activity of antioxidant enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxide content, and lipid peroxidation (hydrogen peroxide H_2O_2 and malonaldehyde MDA). Carrot extract increased the germination rate and maintained germination even under increased salinity rates in both cultivars. The application of 25 mM NaCl also boosted germination rates, followed by a significant decrease due to increased salinity rates. Shoot and root lengths and dry mass parameters showed a linear decrease in response to increasing NaCl concentrations. The activities of superoxide dismutase (SOD), ascorbate peroxidase (APx), and catalase (CAT) enzymes tended to decrease as the concentration of carrot extract increased, whereas the opposite was observed with NaCl application. Based on the combined analysis of the evaluated parameters, carrot extract application under the tested conditions was efficient in mitigating oxidative stress caused by high salinity conditions.

Keywords: *Oryza sativa* L.; *Daucus carota*; salinity; germination; growth; oxidative stress

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1. Introduction

The impact that soil salinization has had on modern agriculture is a major challenge and has considerable impacts on global food security and environmental sustainability. In addition, the harmful impacts of climate change are exacerbating the impact of this stress, triggering an even greater problem in regions that have not yet been affected [1,2]

In agriculture, abiotic stressors are non-living environmental conditions that inhibit plant growth and reduce agricultural productivity [3]. In addition to soil salinity, drought, extreme temperatures, flooding, insufficient nutrients, heavy metal toxicity, ultraviolet radiation, air pollution, and wind severely threaten agricultural practices [4]. High levels of salinity impair plant germination and growth, interfere with various physiological processes and nutrient uptake, and decrease crop yield and quality [5,6]. Even for crops such as rice, which already have many resistant varieties, salt stress can be considered a problem, especially in the seedling and flowering phases, which are considered more critical [5]. This is because rice seedlings die at a salt level of 10 dSm^{-1} (100 mM) [7], and yield losses can reach 90% at a salt level of 3.5 dSm^{-1} in the reproductive phase [8]. In addition, salinity can alter enzymatic activity and nucleic acid metabolism, mainly due to the production of reactive oxygen species (ROS) [9,10], causing late oxidative damage and loss of crop yields. Given the widespread degradation of agricultural areas, coupled with uncertainties related to climate change, biostimulants are an intriguing option to help meet the global demand for maintaining food production by increasing the yield and quality while mitigating environmental stresses [11].

Higher plants, responsible for natural defense against biotic and abiotic stress, produce a wide variety of secondary metabolites [12]. MacDonald et al. [13] demonstrated that β-carotene, vitamins C and E, and phenolic compounds are associated with the antioxidant capacities of several vegetables. Among these vegetables, carrot roots contain a wide variety of high-value compounds, including phenolic compounds such as coumarins [14] and p-hydroxybenzoic acid [15], volatile terpenoids [16], and several isoprenoid compounds like carotenoids [17], phytosterols [18] chlorophylls [19], and tocopherols [20] that may play an important role in protecting against salt stress. These antioxidant compounds can help reduce oxidative damage caused by saline stress and improve plant resilience.

Carrots are a natural and accessible source of substances that can be used in phytochemical treatments to promote plant protection and recovery. Thus, increasing the induction of enzymatic antioxidant levels through the application of elicitor compounds is a potential mechanism to increase tolerance to saline stress, as plants naturally trigger mechanisms to respond to stress, leading to physiological and biochemical changes. The elucidation of these mechanisms can drive the development of chemically and environmentally accessible formulations, leading to more sustainable mitigation strategies for agriculture, especially in regions with saline soils or where irrigation with saline water is necessary. In this context, carrot extract may show efficacy in improving germination, growth, and activity of antioxidant enzymes, and can be used in plant biostimulation programs or as a natural phytochemical treatment for crops that are sensitive to salinity, helping plants adapt to abiotic stress conditions. This study could lead to discoveries that reduce the reliance on synthetic chemicals and fertilizers that have historically been used, offering a more sustainable and environmentally friendly approach to agricultural management.

Thus, the objective of the present study was to investigate (i) the biostimulation and bioprotective effects of conditioning rice seeds with an aqueous extract of carrot roots on seed germination and (ii) the biochemical responses of rice seedlings from osmoprimed seeds treated with carrot root extract under saline stress conditions.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The experiment was conducted at the Seed Physiology Laboratory of the Biology Institute of the Federal University of Pelotas (UFPel) in Capão do Leão County, Rio Grande do Sul State (RS), Brazil. Seeds of the irrigated rice cultivars BRS Querência (indica subspecies) and BRS 358 (japonica subspecies) were used in the trials.

To obtain the extract, freshly harvested carrots, and their roots were cleaned, crushed, and centrifuged (model Mondial premium) on the day of its use. The resulting extract was diluted in distilled water to obtain different established percentages, which were characterized by pH, osmotic potential (Ψs), and electrical conductivity (EC) (Table 1). The obtained extract was diluted in water to create solutions of 0% (no extract), 25%, 50%, and 100% (*v*/*v*) carrot extract [21]. The selected seeds were disinfected with 2% sodium hypochlorite for 5 min. Subsequently, they were osmoconditioned for 48 h using carrot root extract. After the imbibition period, seeds were subjected to 0 (control), 25, 75, and 150 mM NaCl treatments during the tests.

Table 1. Characterization of the carrot root extract as pH, osmotic potential (Ψs), and electrical conductivity (EC).

Carrot Extract (%)	pH	Ψs (MPa)	EC (mS cm^{-1})
25	6.04	−0.13	1.19
50	6.04	−0.31	1.98
100	6.04	−0.61	3.63

2.2. Germination and Seedling Performance Evaluation

A germination test was conducted to determine the number of germinated seeds according to the Seed Analysis Rules [22]. The test was carried out with 200 seeds grown on germitest paper moistened with NaCl solutions at concentrations of 0, 25, 75, and 150 mM, with the solution volume being 2.5 times the weight of the paper. The samples were kept in a germinator at 25 °C under a 12-h photoperiod. Following the Seed Analysis Rules, the percentage of normal seedlings was assessed 14 d after sowing.

Shoot and root lengths and dry mass were measured based on an average of 10 seedlings at the end of the germination test. Shoot and root lengths were measured using a millimeter ruler and recorded in centimeters (cm). Dry mass was determined gravimetrically using a precision scale after drying the plant material in an oven at 70 ± 1 °C until a constant mass was achieved; results were reported in milligrams (mg).

2.3. Determination of Antioxidant Enzyme Activity

The seedlings (200 mg) were ground into powder using 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM ascorbic acid (AsA), and 50% (*w*/*w*) polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 12,000× *g* at 4 °C for 20 min, and the supernatant was used for enzyme activity assays. Protein quantification was performed according to the method described by Bradford [23].

The superoxide dismutase activity was measured based on its ability to inhibit nitroblue tetrazolium (NBT) [24]. The reaction medium contained 50 mM potassium phosphate (pH 7.8), 14 mM methionine, 0.1 mM EDTA, 75 µM NBT, and 2 mM riboflavin. Readings were taken using a spectrophotometer (model Shimadzu Europa GmbH Albert-Hahn-Strasse 6-10, 47269 Duisburg, F.R. Germany) at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to achieve 50% inhibition of NBT photoreduction [25]. Catalase activity was determined according to Azevedo et al. [26] with modifications. The decrease in absorbance was measured at 240 nm for 90 s at 25 °C. The reaction medium, incubated at 34 °C, contained 12.5 mM H_2O_2 and an enzyme extract. Measurements were recorded using a spectrophotometer (model Ultrospec 7000). Additionally, ascorbate peroxidase activity was measured according to Nakano and Asada [27], with modifications, by evaluating the rate of ascorbate oxidation. The reaction medium consisted of 100 mM potassium phosphate buffer (pH 7.0) and 0.5 mM ascorbic acid and was incubated at 34 °C. H_2O_2 (0.1 mM) and the enzyme sample were then added. Ab-

sorbance decrease was monitored at 290 nm for 1 min and 30 s. The results are expressed as μmol ASA min^{-1} mg $protein^{-1}$.

2.4. Determination of Peroxide Content and Lipid Peroxidation

Peroxide content and lipid peroxidation were assessed in approximately 200 mg of shoot fresh mass (FM). Seedlings were macerated in N_2 and mixed with 20% PVPP and 2 mL of an extraction solution containing 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000× *g* for 20 min, and the supernatant was collected for further analysis.

Lipid peroxidation was determined by quantifying thiobarbituric acid reactive species (TBARS), as described by Cakmak and Horst [28]. Thiobarbituric acid (TBA) forms reddish complexes with low-molecular-weight aldehydes, such as malondialdehyde (MDA), a secondary product of lipid peroxidation. The concentration of the MDA/TBA complex was calculated using the following equation: [MDA] = (A535 − A600)/(ξ.b), where ξ is the extinction coefficient (1.56×10^{-5} cm^{-1}), and b is the optical length (1 cm). Lipid peroxidation was expressed as μmol g^{-1} FM.

Hydrogen peroxide (H_2O_2) content was quantified according to the method described by Velikova, Yordanov, and Edreva [29]. The concentration of H_2O_2 was measured using a spectrophotometer (model Ultrospec 7000) at 390 nm and expressed in nmol g^{-1} FM.

2.5. Statistical Analysis

The study followed a completely randomized design with a 4 × 4 factorial arrangement (4 extract conditions × 4 salt concentrations) and three replicates per treatment. Data were analyzed using multiple regression, and the response surfaces were adjusted according to the extract and NaCl concentrations. For data that did not conform to the response surface model, polynomial regression analysis was performed for the conditioning factors, such as salinity, at a 5% probability. All statistical analyses were conducted using SigmaPlot 12.5 software.

3. Results

3.1. Seed Germination and Seedling Performance

Germination (G) exhibited a quadratic response to the factors assessed in this study, as shown by the response surface analysis. For cultivar BRS Querência, the maximum germination was obtained (98.5%) with 100% extract and 25 mM NaCl. The application of carrot extracts enhanced germination, and higher concentrations of the extract maintained germinative ability, even with the addition of salt. This result was significantly different from that of the control (Figure 1A). For cultivar BRS 358, the highest germination occurred at 25 mM NaCl with 50% carrot extract (Figure 1B) and with the application of pure carrot extract (100%). However, germination rates decreased as salinity levels increased.

The results obtained for the shoot length (SL) and dry mass (SDM) of the root of the Querencia cultivar showed the highest average in 25% extract and 25 mM NaCl (4.4 cm), with a gradual reduction of this parameter being observed with the increase in stress and with the maintenance of the length similar to the control in the plants that were subjected to the extract, even under saline stress conditions. (Figure 2A,C). Salinity negatively impacted the plant shoots, mainly in the control group (0% extract) with 100% carrot extract. Root length (RL) and root dry mass (RDM) were not significantly influenced by carrot extract; nevertheless, these variables showed a 58.6% and 33% decrease, respectively, with increasing salt concentration (Figure 2B,D).

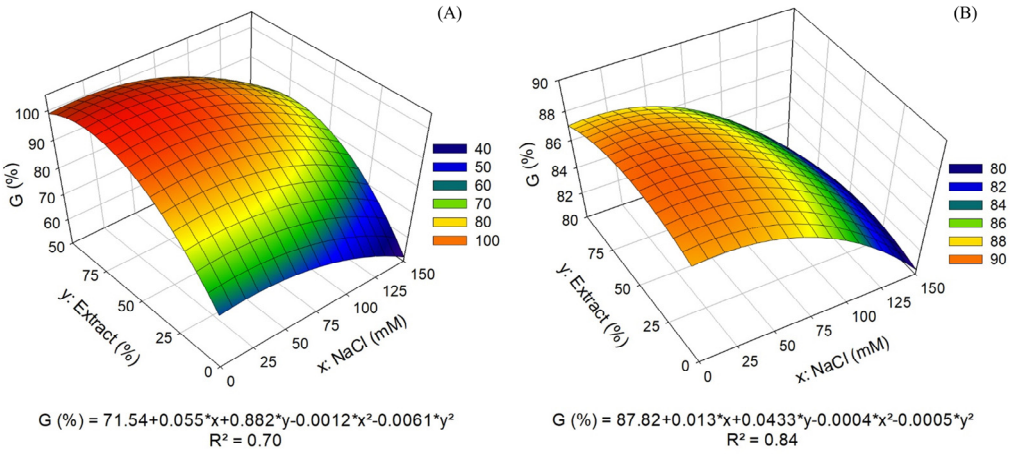


Figure 1. Germination rate (G %) of rice seeds, cultivars BRS Querência (**A**) and BRS 358 (**B**) depending on carrot extract (y) and subjected to different NaCl concentrations (x).

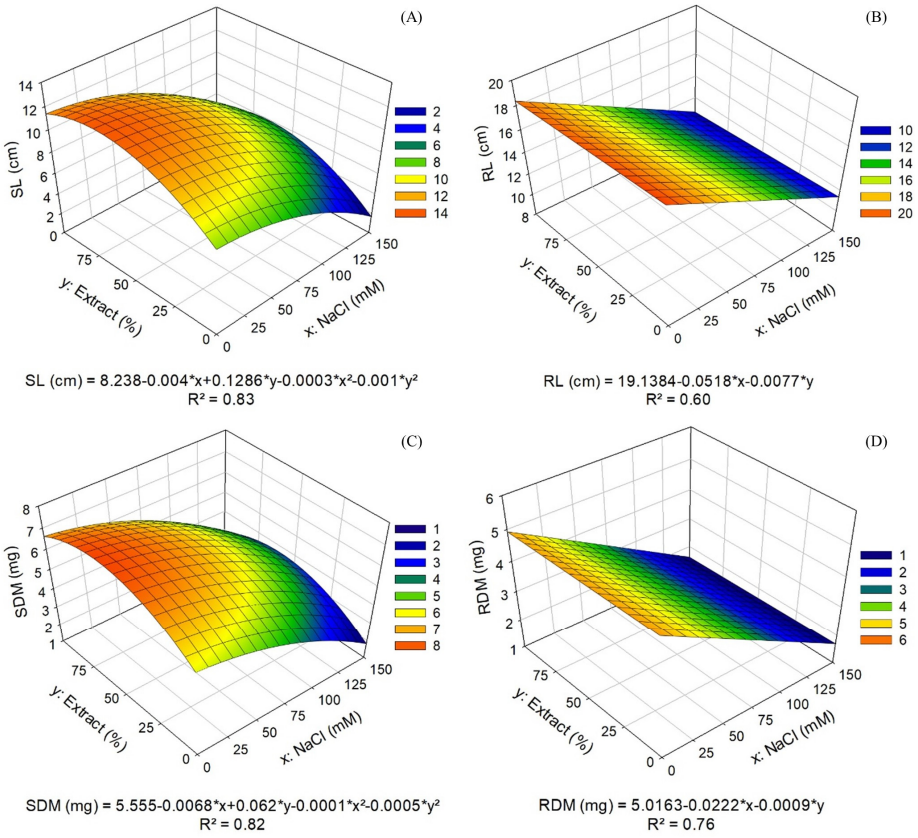


Figure 2. Shoot length (SL) (**A**), root length (RL) (**B**), shoot dry mass (SDM) (**C**), and root dry mass (RDM) (**D**) of BRS Querência rice seedlings depending on the carrot extract rate (y) and subjected to different NaCl concentrations (x).

Shoot length (SL) in cultivar BRS 358 did not respond to carrot extract application but showed a 64.4% reduction when NaCl concentrations were increased from 0 mM to 150 mM (Figure 3A). Shoot dry mass (SDM) responded to both factors, reaching a maximum of 33 mM NaCl under 58% carrot extract; however, the best results were observed under lower extract rates combined with higher salt concentrations (Figure 3C). Root length (RL) increased with higher extract rates, achieving maximum length at 50 mM NaCl with 100% carrot extract. RL also decreased with increasing salinity, particularly in the control group. Root dry mass (RDM) significantly responded to carrot extract applications, reaching its peak at 21 mM NaCl with 92% carrot extract.

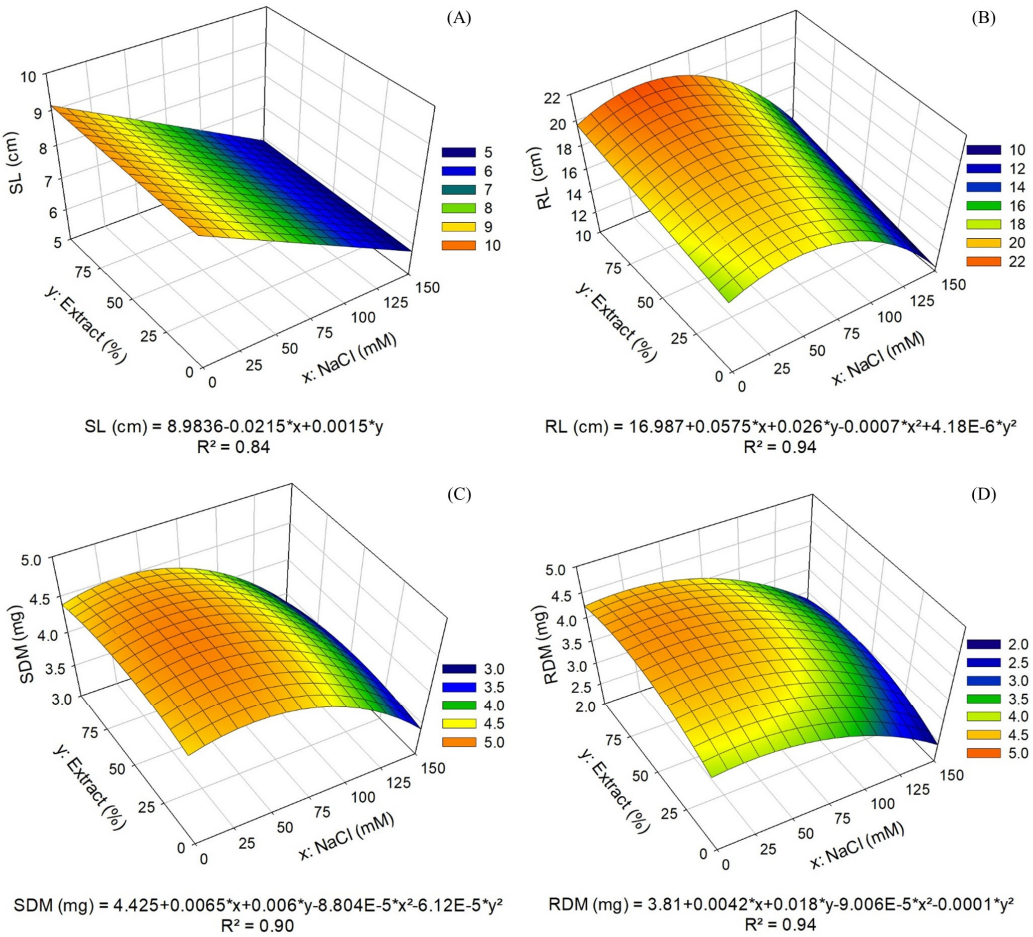


Figure 3. Shoot length (SL) (**A**), root length (RL) (**B**), shoot dry mass (SDM) (**C**), and root dry mass (RDM) (**D**) of BRS 358 rice seedlings depending on the carrot extract rate (y) and subjected to different NaCl concentrations (x).

3.2. Activity of Antioxidant Enzymes

The SOD activity in the shoots of cultivar BRS Querência seedlings was influenced by the interaction between carrot extract rate and NaCl concentration. Conversely, no significant effect was observed when carrot extract was used in seeds germinated in water (0 mM NaCl). However, 25 mM NaCl increased the SOD activity by 1.19 U mg^{-1} protein for each percentage (%) of extract addition (EA) (Figure 4A). There was a quadratic response to extract application in the 75 mM NaCl treatment, which led to a peak of 579.48 U mg^{-1} of protein with 44.5% carrot extract. The response to extract application during germination with 150 mM NaCl was adjusted to a quadratic positive model, which recorded a minimum enzyme activity of 390 U mg^{-1} protein at 48.5% carrot extract.

Regarding salinity, none of the seedlings (0%) exhibited minimal specific activity when germinated in 72 mM NaCl (Figure 4B). In contrast, treatments with 25% and 50% extract showed a quadratic positive behavior, with maximum activity at 102 mM and 45 mM NaCl, respectively. Treatment with 100% carrot extract did not elicit a response to different NaCl concentrations.

Ascorbate peroxidase (APX) activity responded to the interactions between the analyzed factors. APX activity was adjusted to a linear negative model for the control group, decreasing at a rate of 0.091 mmol ASA min^{-1} mg^{-1} protein per EA (%). Carrot extract application at a rate of 0.182 mmol ASA min^{-1} mg^{-1} protein per EA (%) reduced APX activity at 75 mM NaCl. Enzyme activity was adjusted to a quadratic positive model at 150 mM NaCl, reaching a minimum of 19.36 mmol ASA min^{-1} mg^{-1} protein under 58%

carrot extract. This enzyme did not respond to carrot extract application at 25 mM NaCl (Figure 4C), except for treatments with 0% and 100% carrot extract, which responded to the increased saline dose. None of the seedlings (0%) showed increased APX activity at a rate of 0.076 mmol ASA min^{-1} mg^{-1} protein per mM NaCl. Treatment with 100% carrot extract reached the minimum activity point at 49 mM NaCl (Figure 4D).

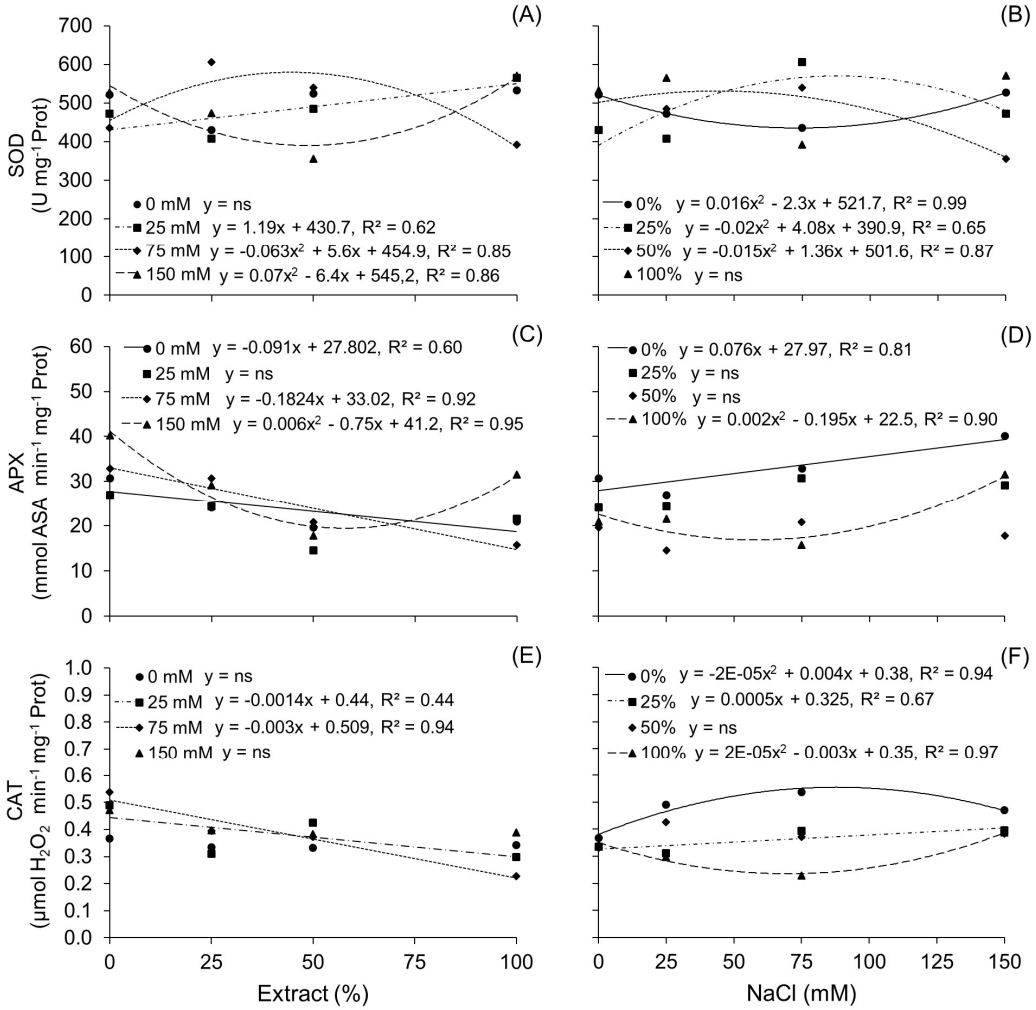


Figure 4. Superoxide dismutase (**A**,**B**), ascorbate peroxidase (**C**,**D**), and catalase (**E**,**F**) activities in the shoots of rice seedlings of cv. BRS Querência, osmoconditioned with different doses of carrot extract (**A**,**C**,**E**) and subjected to different NaCl concentrations (**B**,**D**,**F**).

There was a linear reduction in CAT activity due to carrot extract application in cv. BRS Querência when seedlings were subjected to NaCl concentrations of 25 mM and 75 mM, with reduction rates of 0.0014 and 0.0029 µmol H_2O_2 min^{-1} mg^{-1} protein per EA (%), respectively (Figure 4E). The other salt concentrations did not affect CAT activity. Catalase activity increased due to salinity in the treatment with 25% carrot extract. Treatment with 0% carrot extract showed a quadratic negative effect, with maximum activity at 100 mM NaCl, while seedlings treated with 100% extract exhibited lower activity at 75 mM NaCl (Figure 4F). Treatment with 50% extract was unresponsive to salinity.

Among the NaCl concentrations tested, only 75 mM NaCl showed an effect of extract concentrations on SOD activity in cv. BRS 358, reaching a minimum at 48.7% extract, with an activity of 159.74 U mg^{-1} protein. The specific activity recorded was 0.142 U mg^{-1} protein per unit of mM NaCl added to the germination medium (Figure 5A,B).

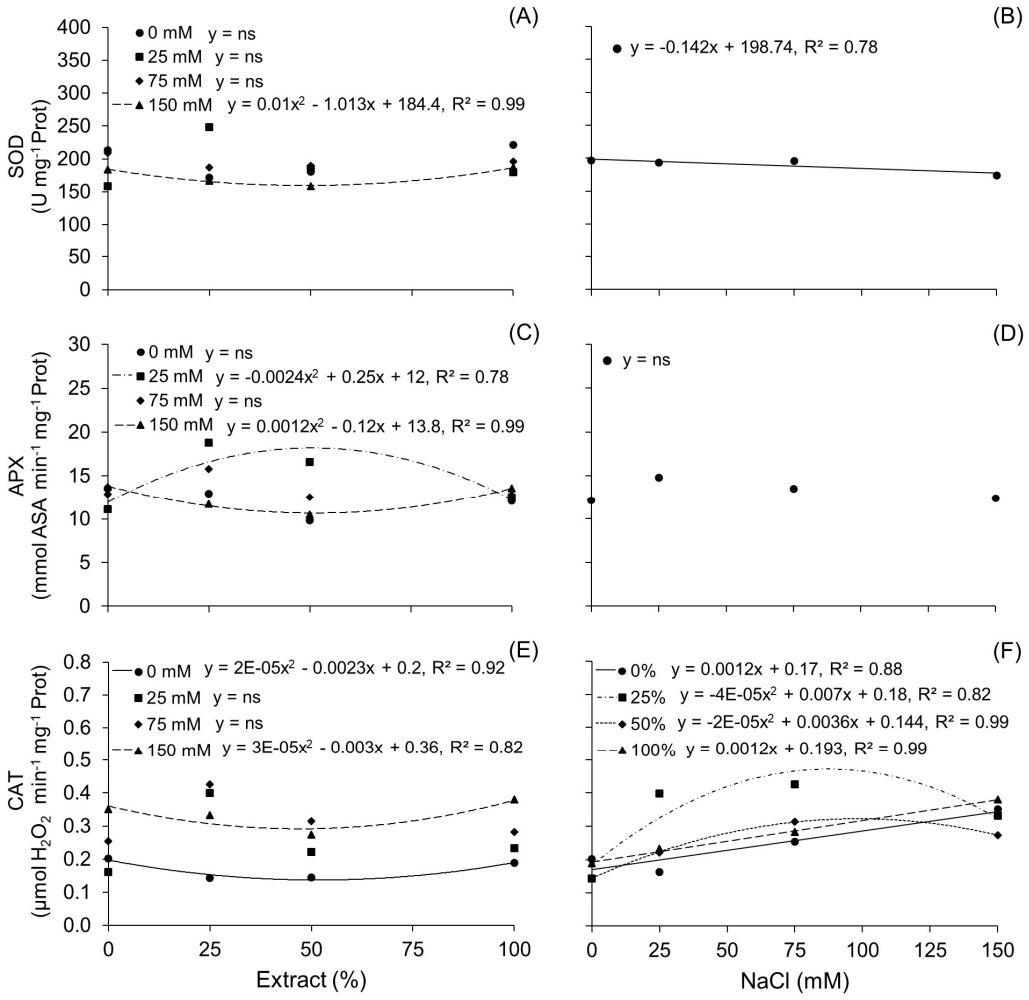


Figure 5. Superoxide dismutase (**A**,**B**), ascorbate peroxidase (**C**,**D**), and catalase (**E**,**F**) activities in the shoots of rice seedlings of cv. BRS 358 osmoconditioned with different doses of carrot extract (**A**,**C**,**E**) and subjected to different NaCl concentrations (**B**,**D**,**F**).

A comparison of means (Table 2) showed that APX activity significantly increased at 100 mM extract and 150 mM NaCl when compared to lower concentrations of extract and NaCl. The same trend was observed for CAT; however, in this same condition, there was no statistically significant difference when compared to 25 mM NaCl (Table 2).

APX activity only showed an interaction between factors at 25 and 150 mM NaCl. It exhibited a quadratic effect with a maximum activity of 18.29 mmol ASA min^{-1} mg^{-1} protein at 25 mM NaCl and a minimum of 10.66 mmol ASA min^{-1} mg^{-1} protein at 150 mM NaCl, both at 51.5% extract. This enzyme was not influenced by other salt concentrations (Figure 5C,D).

Both the control and 150 mM NaCl concentration followed a positive quadratic model for CAT activity, with minimum activity points of 0.131 and 0.290 µmol H_2O_2 min^{-1} mg^{-1} under 57.5% and 48.3% carrot extract, respectively. The other saline concentrations did not fit any of the tested mathematical models (Figure 5E).

Catalase activity was strongly influenced by the NaCl concentration under all conditions. It exhibited a linear increase due to salinity in treatments with 0% and 100% extract, at a rate of 0.0012 µmol H_2O_2 min^{-1} mg^{-1} protein per unit of mM NaCl added to the germination medium (Figure 5F). Treatment with 25% and 50% extract followed a quadratic negative model, with maximum activity points at 87.5 mM and 90 mM NaCl, respectively. CAT activity was also demonstrated in the average test, where a significant increase was observed at 100% extract concentration at 75 and 150 mM NaCl. The variations were subtle

when comparing the different extract concentrations with lower levels of saline stress (Table 3).

Table 2. Enzyme activity of seedlings from rice seeds (Querencia cv.) subjected to different concentrations of carrot extract and subsequent saline stress.

	NaCl (mM)	Carrot Extract Concentration (%)				Average	SE	CV (%)
		0	25	50	100			
SOD	0	521.98	430.24	524.15	532.58	502.20 ^{ns}	4.82	10.61
	25	472.73	407.80	485.33	565.63	482.90 ^{ns}		
	75	435.97	605.91	539.74	392.41	493.50 ^{ns}		
	150	527.67	473.09	355.20	571.20	481.80 ^{ns}		
APX	0	30.75 ^{ab}	24.06 ^{a}	19.57 ^{a}	20.95 ^{b}	23.83	1.66	19.37
	25	26.97 ^{b}	24.25 ^{a}	14.59 ^{a}	21.59 ^{b}	21.85		
	75	32.92 ^{ab}	30.65 ^{a}	20.80 ^{a}	15.78 ^{b}	25.04		
	150	40.19 ^{a}	29.12 ^{a}	17.84 ^{a}	31.49 ^{a}	29.66		
CAT	0	0.37 ^{b}	0.34 ^{a}	0.33 ^{a}	0.34 ^{ab}	0.34	0.01	13.83
	25	0.49 ^{a}	0.31 ^{a}	0.43 ^{a}	0.30 ^{ab}	0.38		
	75	0.54 ^{a}	0.40 ^{a}	0.37 ^{a}	0.23 ^{b}	0.38		
	150	0.47 ^{a}	0.40 ^{a}	0.38 ^{a}	0.39 ^{a}	0.41		

Means were compared using Tukey's test for multiple comparisons, following a one-way ANOVA. Values of p less than 0.05 indicate statistically significant differences between treatments. Different letters represent significantly different means (Tukey's test, $p \leq 0.05$, $n = 4n$); "ns" indicates non-significant differences. SE: Standard error; CV: Coefficient of variation.

Table 3. Enzyme activity of seedlings from rice seeds (BRS 358) subjected to different concentrations of carrot extract and subsequent saline stress.

	NaCl (mM)	Carrot Extract Concentration (%)				Average	SE	CV (%)
		0	25	50	100			
SOD	0	213.20	171.80	180.4	221.63	196.80 ^{ns}	5.32	13.8
	25	158.82	248.49	185.6	179.50	193.10 ^{ns}		
	75	209.10	187.20	189.6	196.38	195.60 ^{ns}		
	150	184.03	166.48	158.9	186.77	174.10 ^{ns}		
CAT	0	0.20 ^{b}	0.14 ^{b}	0.14 ^{b}	0.19 ^{b}	0.17	0.04	16.8
	25	0.16 ^{b}	0.40 ^{a}	0.22 ^{ab}	0.23 ^{b}	0.25		
	75	0.25 ^{ab}	0.42 ^{a}	0.31 ^{a}	0.28 ^{ab}	0.32		
	150	0.35 ^{a}	0.33 ^{a}	0.27 ^{a}	0.38 ^{a}	0.33		
APX	0	13.59 ^{a}	12.94 ^{b}	9.82 ^{b}	12.10 ^{a}	12.12	0.60	24.5
	25	11.14 ^{a}	18.79 ^{a}	16.51 ^{a}	12.53 ^{a}	14.76		
	75	12.83 ^{a}	15.81 ^{ab}	12.55 ^{ab}	12.79 ^{a}	13.51		
	150	13.77 ^{a}	11.71 ^{b}	10.53 ^{b}	13.58 ^{a}	12.40		

Means were compared using Tukey's test for multiple comparisons following a one-way ANOVA. Values of p less than 0.05 indicate statistically significant differences between treatments. Different letters represent significantly different means (Tukey's test, $p \leq 0.05$, $n = 4n$); "ns" indicates non-significant differences. SE: Standard error, CV: Coefficient of variation.

3.3. H_2O_2 Content and Lipid Peroxidation

The H_2O_2 content in the shoots of rice seedlings cv. BRS Querência reflected responses to the interaction between factors. The control exhibited a quadratic negative response to carrot extract application, reaching a maximum of 2.49 mmol H_2O_2 g^{-1} FM at 56.8% extract (Figure 6A). The H_2O_2 content decreased at 0.011 mmol H_2O_2 g^{-1} FM per EA (%) and 25 mM NaCl. This variable was adjusted to a quadratic positive model, with a minimum point of 1.13 mmol of H_2O_2 g^{-1} FM at 43.5% extract and 75 mM NaCl. A similar response

was observed at 150 mM NaCl, reaching a minimum of 0.83 mmol H_2O_2 g^{-1} FM with 62.2% extract (Figure 6A). Treatment with 25% extract reduced the H_2O_2 content at a rate of 0.0112 mmol of H_2O_2 g^{-1} FM per mM of NaCl added due to salinity. In comparison, treatments with 50% and 100% extract recorded minimum content at 75 mM and maximum content at 100 mM NaCl, respectively. Treatment with 0% extract did not elicit any response to salinity (Figure 6B). When lipid peroxidation was analyzed in 100% extract and 150 mM NaCl, a statistical difference in relation to the lowest saline concentration was observed, with an increase in the MDA content in 100 and 25% extracts, directly proportional to the peroxide content in this same condition (Table 4).

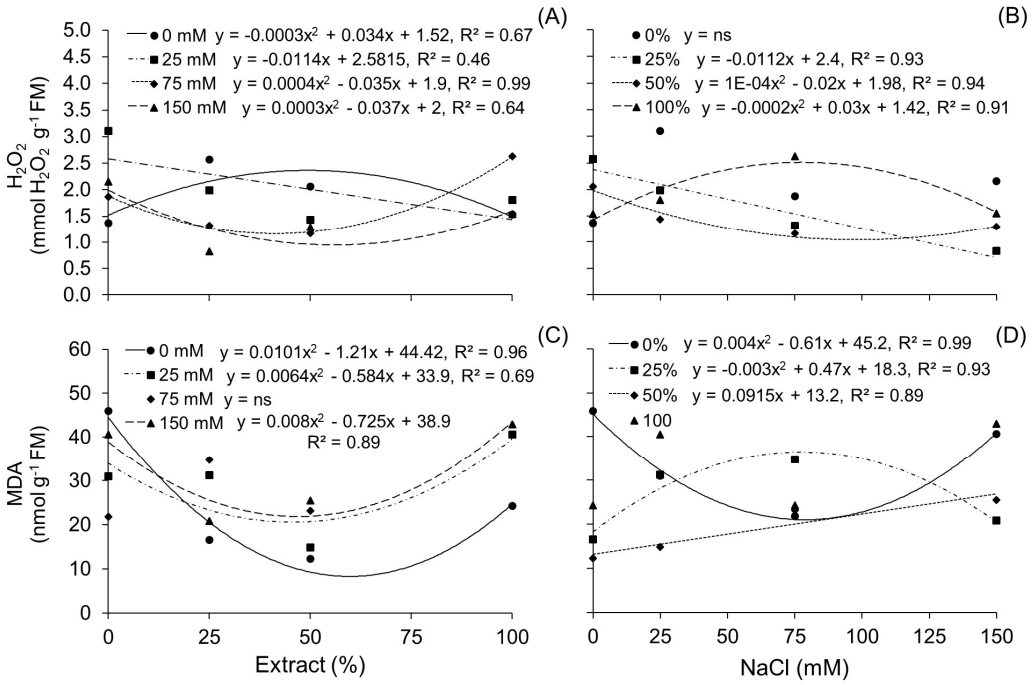


Figure 6. H_2O_2 content (**A**,**B**) and lipid peroxidation (MDA) (**C**,**D**) in the shoot of rice seedlings of cv. BRS Querência osmoconditioned with different doses of carrot extract (**A**,**C**) and subjected to different NaCl concentrations (**B**,**D**).

Table 4. Hydrogen peroxide content (H_2O_2) and Lipidic peroxidation (MDA) of seedlings from rice seeds (Querencia cv.) subjected to different concentrations of carrot extract and subsequent saline stress.

	NaCl (mM)	Carrot Extract Concentration (%)				Average	SE	CV (%)
		0	25	50	100			
H_2O_2	0	1.37 ^{c}	2.57 ^{a}	2.06 ^{a}	1.54 ^{b}	1.88	0.13	12.77
	25	3.10 ^{a}	1.98 ^{b}	1.43 ^{b}	1.80 ^{b}	2.08		
	75	1.87 ^{b}	1.31 ^{c}	1.16 ^{b}	2.62 ^{a}	1.74		
	150	2.15 ^{b}	0.82 ^{d}	1.29 ^{b}	1.54 ^{b}	1.45		
MDA	0	45.90 ^{a}	16.56 ^{b}	12.22 ^{b}	24.17 ^{b}	24.71	1.75	15.81
	25	30.90 ^{b}	31.18 ^{a}	14.76 ^{b}	40.53 ^{a}	29.34		
	75	21.79 ^{c}	34.77 ^{a}	23.12 ^{a}	24.24 ^{b}	25.98		
	150	40.64 ^{a}	20.80 ^{b}	25.48 ^{a}	42.93 ^{a}	32.46		

Means were compared using Tukey's test for multiple comparisons following a one-way ANOVA. Values of p less than 0.05 indicate statistically significant differences between treatments. Different letters represent significantly different means (Tukey's test, $p \leq 0.05$, $n = 4n$); "ns" indicates non-significant differences. SE: Standard error, CV: Coefficient of variation.

There was no interaction between factors at 75 mM NaCl in the lipid peroxidation (MDA) of cv. BRS Querência. Other salt concentrations exhibited a quadratic positive response with minimum MDA values of 8.24 nmol mg^{-1} FM under 60% extract, 20.54 nmol mg^{-1} FM under 45.6% extract, and 21.8 nmol mg^{-1} FM under 47% extract due to water addition at 25 mM and 150 mM NaCl, respectively (Figure 6C). Treatment with 50% extract showed an increase of 0.0915 mmol H_2O_2 g^{-1} FM per mM NaCl due to salinity. Treatment with 0% carrot extract recorded the lowest MDA content at 76 mM NaCl, which was also observed in the 50% extract treatment at the same concentration. Treatment with 25% extract reached the maximum MDA level at 78 mM NaCl (Figure 6D).

Cultivar BRS 358 only showed an interaction between factors at 25 mM NaCl concerning H_2O_2 content, which decreased with carrot extract application at a rate of 0.0008 mmol H_2O_2 g^{-1} FM per EA (%). Only treatments with 25% and 100% carrot extract exhibited lower H_2O_2 content at 120 mM and 69 mM NaCl, respectively (Figure 7A,B). The peroxide content did not show a statistical difference between treatments (NaCl) when 100% extract was used, even though a decrease of 58.47% was observed on average at 25 mM NaCl. Lipid peroxidation showed a significant increase at 100% extract with a significant reduction only at 25% NaCl (Table 5).

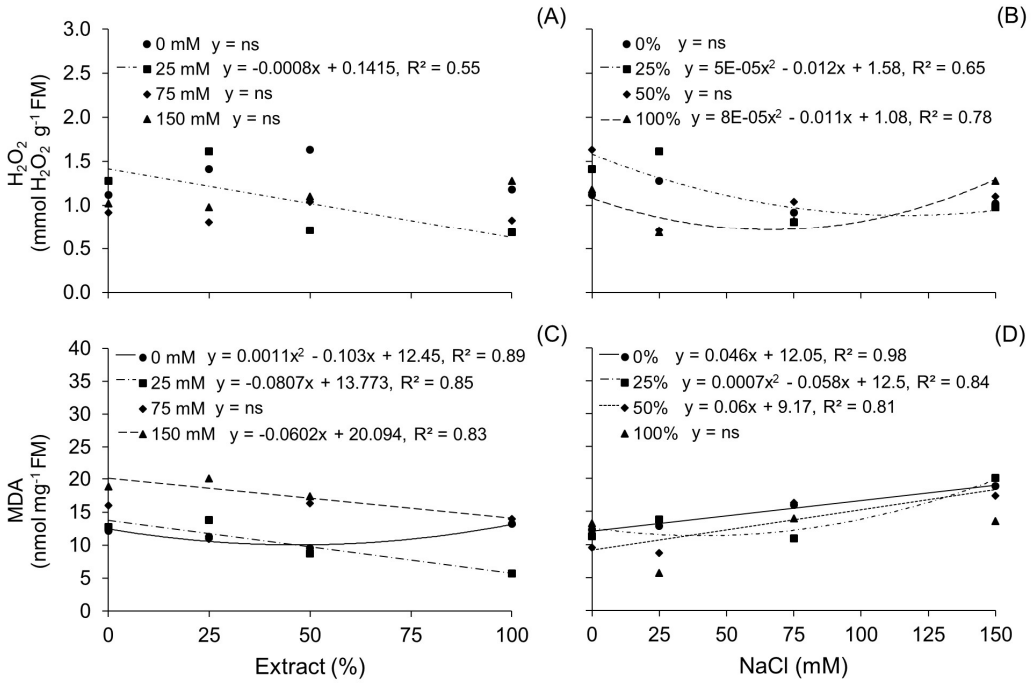


Figure 7. H_2O_2 content (**A**,**B**) and lipid peroxidation (MDA) (**C**,**D**) in the shoot of rice seedlings of cv. BRS 358, osmoconditioned with different doses of carrot extract (**A**,**C**) and subjected to different NaCl concentrations (**B**,**D**).

Control (0 mM) exhibited a quadratic positive behavior for lipid peroxidation (MDA), with a minimum point of 10.04 nmol mg^{-1} FM at 46.8% extract in cv. BRS 358 (Figure 7C). MDA content decreased with increasing extract percentages at 25 mM and 150 mM NaCl, with rates of decrease of 0.08 and 0.06 nmol mg^{-1} FM per EA (%), respectively. Treatments with 0% and 50% extract showed a linear increase in MDA content from 0 to 150 mM NaCl, while the treatment with 25% extract reached its minimum at 41 mM NaCl.

Table 5. Hydrogen peroxide content (H_2O_2) and Lipidic peroxidation (MDA) of seedlings from rice seeds (BRS 358) subjected to different concentrations of carrot extract and subsequent saline stress.

	NaCl (mM)	Carrot Extract Concentration (%)				Average	SE	CV (%)
		0	25	50	100			
H_2O_2	0	1.12 ^{a}	1.41 ^{ab}	1.63 ^{a}	1.18 ^{a}	0.13	0.01	27.3
	25	1.26 ^{a}	1.61 ^{a}	0.71 ^{b}	0.69 ^{a}	0.11		
	75	0.92 ^{a}	0.81 ^{b}	1.04 ^{ab}	0.82 ^{a}	0.09		
	150	1.02 ^{a}	0.98 ^{b}	1.10 ^{ab}	1.28 ^{a}	0.11		
MDA	0	12.19 ^{b}	11.25 ^{b}	9.53 ^{b}	13.2 ^{a}	11.55	1.60	13.2
	25	12.76 ^{b}	13.79 ^{b}	8.71 ^{b}	5.71 ^{b}	10.24		
	75	15.99 ab	10.90 ^{b}	16.34 ^{a}	13.92 ^{a}	14.28		
	150	18.84 ^{a}	20.08 ^{a}	17.36 ^{a}	13.56 ^{a}	17.46		

Means were compared using Tukey's test for multiple comparisons following a one-way ANOVA. Values of p less than 0.05 indicate statistically significant differences between treatments. Different letters represent significantly different means (Tukey's test, $p \leq 0.05$, $n = 4n$); "ns" indicates non-significant differences. SE: Standard error, CV: Coefficient of variation.

4. Discussion

Reduced germination and seedling growth in response to increased saline stress (Figures 1–3) can be attributed to the difficulty roots experience in absorbing water due to the high osmotic potential of the medium, which impairs seed imbibition and root elongation [30]. Similar findings have been reported, with a significant decrease in germination observed in rice seeds subjected to salt stress [31,32].

Salt stress reduces the availability of free water in the soil for plants, resulting in a negative water potential. This decrease in water potential leads to specific ionic toxicities and deficiencies, hampers water absorption, and causes nutritional imbalances in plants [33]. Therefore, it is well established that salinity impairs seed germination and seedling growth, with greater sensitivity at the beginning of the sowing period compared to the reproductive phase [34]. The present study shows that treatment with carrot extract significantly mitigated the inhibitory effects of salt stress on the germination of cv. BRS Querência and seedling growth parameters, including root and shoot lengths of both cultivars and the dry mass of cv. BRS 358 (Figures 1A and 2). This outcome can be attributed to the plant's ability to produce various protective compounds under stress conditions. These compounds can influence the growth of other plants by either promoting or inhibiting it [35,36].

Abbas and Akladious [37] reported that soaking cowpea seeds in carrot extracts and NaCl had a stimulatory effect on seedling growth, with a concentration of 25 g 100 mL^{-1} being the most effective in improving length parameters, both in control plants and those subjected to salinity. Çavuşoğlu and Karaferyeli [38] found that the application of *Ginkgo biloba* L. leaf extract mitigated the negative effects of salt stress on barley seed germination, although it did not affect root length or seedling fresh mass. Similarly, the use of *Aloe vera* L. leaf extract in a 0.15 M NaCl medium significantly increased the germination rate, growth, and fresh mass of onion seedlings compared with the control. This enhancement was primarily attributed to the stimulation of mitotic activity in the embryo [39]. However, not all extracts had the same effect on every plant. For example, Aloe vera extract at concentrations of 1–5% increased the germination rate and growth of lentil seedlings, but when diluted to higher concentrations, the same extract had an inhibitory effect on the root length of onion seedlings, regardless of the presence of salt stress. This suggests that the concentration of the extract is a critical factor in the observed results and that there may be significant differences depending on the plant and the stress condition.

Germination is also impaired by the toxic effects of Na^+ and Cl^- ion absorption [40]. This toxicity leads to changes in enzyme activity, consequently, it affects protein

metabolism [41] and causes hormonal imbalance [42]. Additionally, toxicity reduces the utilization of seed reserves [43] and impairs cell division [44]. Imran et al. [45] observed an increased final germination rate, root length, and fresh mass of lentil seedlings germinated with varying concentrations of *Aloe vera* L. leaf extract (from 1 to 5%) compared to the control. However, İlbaş et al. [46] reported that the same extract, at all tested dilutions (2, 5, 10, 20, and 40%), significantly inhibited root length in onion seedlings, regardless of any stress influence.

In the present study, concentrations of 50 and 100% showed greater efficacy in stimulating the initial development processes of rice plants (Figures 1–3). Carrot extract may have contributed to improved seed germination under stress due to the presence of bioactive compounds (carotenoids (such as beta-carotene), antioxidant vitamins (A, C, and E), polyphenols (flavonoids and phenolic acids), amino acids (such as proline and glutamine), and mineral salts (such as calcium, magnesium, and potassium). According to Chen et al. [47], β-carotene constitutes a large portion (60–80%) of the carotenoids in carrots, followed by α-carotene (10–40%) and lutein (1–5%), which help protect against oxidative stress, improve water and nutrient absorption, regulate cell growth, and activate antioxidant mechanisms. These combined effects may have contributed to promoting more efficient germination and healthy seedling development, even when exposed to higher NaCl concentrations (150 mM).

Salt stress reduces plant growth due to factors such as water deficiency and high osmotic pressure. Consequently, a lack of turgor leads to decreased cell elongation [48]. Other studies have also reported reduced growth in rice plants under salt stress, as evidenced by decreased length and dry mass of both shoots and roots. These findings are consistent with those presented in this study [49,50].

Previous studies have shown that certain plants can exhibit inhibitory potential for some species while stimulating others under similar conditions [51,52]. In a study by Teixeira et al. [21], carrot extract was effective in attenuating cold stress, indicating its high potential to protect against abiotic stresses. For instance, Borges et al. [53] reported that the application of castor bean extract reduced the germination and growth of lettuce plants. Conversely, Silva et al. [54] found that while the same extract had no direct effect on the germination of bean plants, it influenced seedling development at higher extract concentrations. In the present study, the application of carrot extract favored the development of rice plants under salt stress.

Preserving an efficient germination process, in which the seed can fully express its developmental potential, is crucial for managing stress throughout the plant's growth phase. This enables the plant defense system to function effectively under both biotic and abiotic stress conditions. The efficiency of the antioxidant system, along with other physiological mechanisms, enhances plant tolerance to stress, reducing the harmful effects of ROS [55]. This is evident from the results of germination and early seedling development, as shown in Figures 1–3.

In the present study, both tested cultivars exhibited increased CAT and APX activity in response to salinity, although this effect was not observed for SOD (Figures 4 and 5). These findings are consistent with those of Kibria et al. [56], who observed increased CAT and APX activity in salt-tolerant rice genotypes as salt concentrations increased, whereas the activity of both enzymes decreased in salt-sensitive plants under similar conditions. Increased activity of antioxidant enzymes has been associated with reduced oxidative damage and enhanced tolerance to salt in tomato, tobacco, and rice plants [57–59]. Previous studies have also shown that increased salinity levels reduce antioxidant enzyme levels in salt-sensitive rice while enhancing CAT activity in salt-tolerant rice [60–62].

The highest H_2O_2 content in the current study was recorded at 25 mM with 0% extract. Such an increase may have resulted from the production of superoxide radicals generated by SOD activity, which subsequently produces H_2O_2 (Tables 3 and 4). Despite this, lipid peroxidation levels remained stable, which can be attributed to the effective action of APX and CAT, which convert H_2O_2 into H_2O and O_2. However, with increased salinity, lipid peroxidation levels rose even without a corresponding increase in H_2O_2 content. This could be attributed to the high levels of superoxide radicals, likely as a consequence of low SOD activity, leading to lipid peroxidation and subsequent membrane damage. The increased levels of MDA content in the present study were caused by salinity (especially at concentrations of 50 and 100 mM NaCl), while H_2O_2 levels increased at 25 mM NaCl but decreased due to the increased salt concentrations.

According to Das et al. [63], salt stress significantly elevates H_2O_2 levels and MDA content in both the roots and shoots of rice seedlings. In the present study, SOD activity increased in the cultivar BRS Querência as the concentration of carrot extract increased, while CAT and APX activities decreased in both cultivars under the same treatment conditions. The reduction in H_2O_2 and MDA levels indicates that carrot extract may have played a role in mitigating lipid peroxidation, likely due to the antioxidant compounds, such as carotenoids and tocopherols, present in the extract (Figures 4 and 5). These findings support the hypothesis that carrot plant extract, through its antioxidant properties, can counteract the oxidative stress induced by elevated NaCl concentrations, thereby enhancing plant stress tolerance.

5. Conclusions

Carrot root extract efficiently attenuates oxidative stress in rice seedlings of the BRS Querência and BRS 358 cultivars subjected to various NaCl concentrations. This extract reduces H_2O_2 and lipid peroxidation levels in seedlings and improves seedling growth parameters.

The use of carrot extract may be the starting point for a promising solution aimed at mitigating the impacts caused by salt stress. In addition to providing insights into the role of carrot extract in protecting against oxidative stress, this study may pave the way for the development of natural and sustainable solutions to increase agricultural productivity in saline soils, thereby contributing to food security and sustainable agriculture.

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