The effect of salt stress on growth parameters of *Oryza sativa* (rice) variety NSIC Rc 442

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Abstract

Salinity is one of the leading causes of crop yield loss worldwide. The presence of harmful cations and anions in the soil through seawater intrusion is the main cause of soil salinization in the Philippines. With this, the study focused on the effect of three types of salts: NaCl, KCl, and $CaCl_2$ on the germination stage of rice. Three different salts with three different concentrations along with a negative control were utilized for the setup. Three replicates were utilized for each treatment. Three Petri dishes were utilized per replicate, each containing fifty seeds. Salt solutions were prepared at three different salinity levels (4, 6, and 8 dS/m). Seeds were allowed to grow for 10 days and germination parameters were measured and recorded thereafter. All data were analyzed using One-way ANOVA at 95% confidence level. Germination percentage showed no significant difference among salts and salinity levels. All the lengths of the shoots and roots, and fresh weights of seeds exposed to NaCl, KCl, and CaCl2 at varying salinity levels showed significant differences.

Keywords: *salt stress, Oryza sativa, sodium chloride, potassium chloride, calcium chloride*

Introduction. The cultivated *Oryza sativa* (rice plant) is a grass of the *Gramineae* family. It is an important crop worldwide, responsible for being the major source of carbohydrates for nearly half of the world's population [1]. It grows in a wide range of environments, particularly centered around Asian countries such as China, India, and the Philippines. Currently, biotic and abiotic stresses such as soil pH, rainfall, salinity, insects, fungi, and diseases are the leading cause of crop failure and decrease in average yield [2]. Of these factors, salinity is among the most widespread problems encountered in rice production worldwide due to its prevalence in coastal fields and irrigations.

For agricultural production, irrigation of the land with salinity is a major menace to modern agriculture, resulting in imbalance and crop failure. According to Vibhuti et al. [2], seed germination decreased from 100% in control to 65% in 20 dS/m. Although most of these results are based on sodium chloride (NaCl), it is hypothesized that other salts have similar effects, but to different degrees [3]. Many studies have used NaCl solutions to study salinity tolerance in the germination of *Oryza sativa* [1,4,5], but little information exists regarding the effect of other salts on the germination of rice seeds.

The most common salts found in irrigation water are the following: sodium chloride (NaCl), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), magnesium sulfate ($MgSO_4$), calcium sulfate ($CaSO_4$), calcium chloride ($CaCl_2$), potassium chloride (KCl), and potassium sulfate (K_2SO_4) [6]. Soils usually have these high concentrations due to a number of factors, including expanding urbanization [7] and seawater intrusion [8]. Although seawater intrusion is the main source of salinity in the Philippine setting, other factors such as agricultural malpractice should be taken into consideration. With this status in the Philippine soils, increasing concentrations of salts may inhibit plant growth especially in its germination period since it is the earliest stage of plant development.

This study aimed to determine the effect of increasing salinity levels of sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2$) on the germination parameters of *Oryza sativa* (rice) variety NSIC Rc 442. It specifically aimed to:

(i) compare the germination percentage, germination rate (also known as the periodic germination percentage), and germination time of rice seeds exposed to different types of salts (NaCl, KCl, and $CaCl_2$) at increasing salinity levels (control, 4 dS/m, 6 dS/m, and 8 dS/m);

(ii) measure the mean length of roots and shoots of 10 seedlings exposed to different types of salts (NaCl, KCl, and $CaCl_2$) at increasing salinity levels (control, 4 dS/m, 6 dS/m, and 8 dS/m) per setup after 10 d;

(iii) measure the fresh weight of shoots and roots of at least 10 seedlings exposed to different types of salts (NaCl, KCl, and $CaCl_2$) at increasing

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salinity levels (control, 4 dS/m, 6 dS/m, and 8 dS/m) after 10 d; and

(iv) compare the leaf color of the seedlings after 10 d.

Methods. Three different salts with three different concentrations, along with a negative control, were utilized for the setup. Three replicates were utilized for each treatment. Three Petri dishes were utilized per replicate, each containing fifty seeds. Salt solutions were prepared at three different salinity levels (4, 6, and 8 dS/m) [9]. Seeds were allowed to grow for 10 days and germination parameters were measured and recorded thereafter. All germination parameters were analyzed using One-way ANOVA at 95% confidence level using SPSS ver. 23.

Seed Acquisition, Pre-germination, and Quality Testing. Seeds of the variety NSIC Rc 442 utilized in the experiment were acquired from Western Visayas Agricultural Research Center (WESVIARC). Moisture content determination was conducted in the same institution. The desired moisture content range was from 8% to 14%. Seeds were then soaked in distilled water for 48 h. Seed quality testing was done by identifying pure and impure seeds [10].

Germination Experiment. Seeds were allowed to germinate for 10 days. Three types of salt and four treatments in each salt type were utilized, namely the control, 4 dS/m, 6 dS/m, and 8 dS/m treatment [9]. In each treatment, there were three replicates with three Petri dishes for each. Fifty seeds were placed in each Petri dish along with 20 mL of their specific salt solutions on day 0 of germination. Each day, 5 mL of distilled water is added. Parameters such as humidity and ambient temperature were recorded thrice a day at 8:00 AM, 12:00 PM, and 4:00 PM. Seeds were exposed to sunlight from 8:00 AM to 4:00 PM, and exposed to artificial light from 4:00 PM to 8:00 AM. Germination parameters (germination rate and time) were recorded accordingly and daily. Germination percentage, length of shoots and roots, fresh weight of shoots and roots, and leaf color were recorded after germination.

Computation of Parameters. Germination percentage and germination rate were solved using the following formulas:

Germination Rate = [(GR_0 x GR_1)/50] x 100; wherein
GR_0 = Total number of germinated seeds today.
GR1 = Total number of germinated seeds yesterday.

Germination Percentage = (TG/TS) x 100; wherein
TG = Total number of germinated seeds.
TS = Total number of tested seeds.

Statistical Analysis. Data were analyzed using one- way Analysis of Variance (ANOVA) at a 95% confidence interval (α = 0.05) performed using Statistical Packages for the Social Sciences (SPSS) version 23. Least Significant Difference Post-Hoc Test was performed after One-way ANOVA.

Safety Procedure. During the implementation, the use of protective equipment such as face masks, nitrile gloves and lab gowns was always observed. For the disposal, all seedlings were washed with running water and disposed inside a black trash bag. All salt solutions were placed inside a plastic bottle, and placed inside the trash bag, alongside the seedlings. All Petri dishes were washed with dishwashing soap, and treated with 70% isopropyl alcohol, then left to dry overnight.

Results and Discussion. Germination percentage was not significantly affected by the type of salt and salinity levels, whereas germination rate decreased with increase in salinity level, regardless of the type of salt. Shoot length under the NaCl and KCl treatments decreased with increasing salinity level. Also, the root length decreased as the salinity level increased. For the $CaCl_2$ treatment, the shortest root and shoot length was at 4 dS/m. Fresh weight of roots and shoots was significantly affected by both the type of salt and salinity levels. Increasing the salinity level resulted in a decrease in the mean fresh weight of roots and shoots.

These findings may be attributed to the high Na^+ and Cl^- concentrations that cause the stomata to be less responsive allowing various plant injuries and growth inhibition. The role of K^+ is necessary for osmoregulation and protein synthesis, maintaining cell turgor and stimulating photosynthesis while externally supplied Ca^{2+} has been shown to ameliorate the adverse effects of salinity especially in osmotic adjustment and growth. Both K^+ and Ca^{2+} are required to maintain the integrity and functioning of cell membranes but would induce stress and injury at toxic levels [11].

Germination Percentage. No significant difference was found between all treatments, including the control (Table 1). All treatments had a germination percentage greater than 90%. Supposedly, increasing salinity levels would show a decrease in the germination percentage [2,4,5]. This opposing result could be due to the qualifications of a seed to be considered "germinated" which requires the main radicle to be at least 2 mm in length.

Germination Rate. The rate of germination was delayed with an increase in salinity level, leading to different germination times. Most seeds treated with

Table 1. The effect of salinity concentration and type of salt on the germination percentage.

Type of Salt	Concentration 4 dS/m	6 dS/m	8 dS/m
		Germination Percentage (%)	
NaCl	96.67 ± 1.11	95.33 ± 1.29	96.89 ± 0.68
KCl	95.78 ± 0.91	94.67 ± 1.53	95.78 ± 1.02
$CaCl_2$	94.67 ± 1.45	96.00 ± 0.82	95.56 ± 1.14
Control	96.67 ± 0.49	96.67 ± 0.49	96.67 ± 0.49
P-value	0.362	0.375	0.601

4 dS/m achieved a 50% germination percentage on the 2nd day while those treated with 8 dS/m achieved the same percentage by the 4th day.

Effects on root and shoot lengths. Root and shoot lengths were significantly affected by salt type and concentration. For seedlings treated with NaCl and KCl, both root and shoot lengths decreased with increasing concentration (Tables 2, 3, & 4). For seedlings under $CaCl_2$, maximum root and shoot length was achieved at 6 dS/m, while the shortest was at 4 dS/m. Reduction of shoot length is a common phenomenon of rice and other crop plants grown in highly saline conditions [12]. The decrease in shoot length is due to the reduction in physiological availability of water with an increase in solute suction or accumulation of toxic ions within the seedlings [13]. Balkan et al. [5] also reported that root length decreased at increasing salinity levels starting from 4 dS/m which might be due to greater inhibitory effects of different salts to root growth [12,14].

Effects on mean fresh weight. Fresh weight of roots and shoots was significantly affected by the type of salt and salinity levels (Tables 2, 3, & 4), which could be attributed to root and shoot lengths of the different treatments. Increasing salinity levels resulted in a decrease in mean fresh weight of roots and shoots, as supported by Balkan et al. [5], Rahman et al. [12], and Vibhuti et al. [2]. Fresh weight of roots and shoots under NaCl and KCl treatments decreased with increasing salinity levels. In the case of $CaCl_2$, the lowest mean fresh weight of roots and shoots was attained at 4 dS/m, followed by 8 dS/m, and 6 dS/m. This shows that different salts have different effects, depending on the type of salt used and its respective concentration.

Limitations. Due to time constraint, this study has only pursued to observe the effects of salinity on germination parameters. There is also a limitation in the objectives of the study, as leaf color was originally part of the results. Due to the 21-day minimum period for valid leaf color results, the use of the leaf color chart provided was not applicable to the seedlings.

Conclusion. The different salts induced significantly stunted the growth of physiological parameters of rice during the germination stage. Also, the degree of this effect increased with salinity concentration, but the extent differed per salt. With increasing salinity concentrations, germination percentage, shoot and root length, and mean fresh weight were adversely affected. For seeds treated with NaCl and KCl, increasing concentrations resulted to a decrease in value of recorded parameters. For seeds treated with $CaCl_2$, seeds showed better performance at 6 dS/m, while it achieved the lowest values at 4 dS/m.

Table 2. The effect of different types of salts at 4 dS/m on the investigated traits.

Salt	Lag (days)	Shoot Length (mm)	Root Length (mm)	Mean Fresh Weight (g)
NaCl	3 days	55.57 ± 3.17 a	24.69 ± 3.39 a	0.2110 ± 0.0222 ab
KCl	3 days	53.38 ± 4.86 a	23.22 ± 3.00 a	0.1900 ± 0.0239 ac
$CaCl_2$	4 days	42.09 ± 2.16 b	6.694 ± 0.77 b	0.1379 ± 0.0100 c
Control	~	77.38 ± 2.21 c	36.49 ± 1.78 c	0.2520 ± 0.0117 b

*Means followed by the same letter are not significantly different at P = 0.05 (LSD Test).

Table 3. The effect of different types of salts at 6 dS/m on the investigated traits.

Salt	Lag (days)	Shoot Length (mm)	Root Length (mm)	Mean Fresh Weight (g)
NaCl	4 days	35.57 ± 3.19 a	12.66 ± 3.10 a	0.1023 ± 0.0127 ab
KCl	3 days	34.67 ± 3.94 a	11.36 ± 1.58 a	0.1257 ± 0.0225 ac
$CaCl_2$	4 days	50.54 ± 1.28 b	12.54 ± 1.44 a	0.1857 ± 0.0126 c
Control	~	77.38 ± 2.21 c	36.49 ± 1.78 b	0.2520 ± 0.0117 b

*Means followed by the same letter are not significantly different at P = 0.05 (LSD Test).

Table 4. The effect of different types of salts at 8 dS/m on the investigated traits.

Salt	Lag (days)	Shoot Length (mm)	Root Length (mm)	Mean Fresh Weight (g)
NaCl	4 days	23.12 ± 1.42 a	5.389 ± 1.09 a	0.0681 ± 0.0106 a
KCl	4 days	18.10 ± 0.70 a	5.072 ± 0.27 a	0.0541 ± 0.0064 a
$CaCl_2$	4 days	46.07 ± 2.13 b	7.989 ± 0.63 a	0.1634 ± 0.0162 b
Control	~	77.38 ± 2.21 c	36.49 ± 1.78 b	0.2520 ± 0.0117 c

*Means followed by the same letter are not significantly different at P = 0.05 (LSD Test).

Recommendations. It is recommended that the setup will have more number of salts and salinity levels to utilize. It would also be best if the study is conducted in a soil setup and would be extended until yield to further imitate the natural environment and to measure more parameters for more conclusive data.

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