Bulgarian Chemical Communications, Volume 56, Special Issue D1 (pp. 49-54) 2024 DOI: 10.34049/bcc.56.D.S1P10

The effects of NaCl, KCl, $CaCl_2$ and $MgCl_2$ on the germination and seedling growth of *Oryza sativa* L. seeds

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Received: November 3, 2023; Revised: April 09, 2024

The effect of increasing concentrations (50, 100, 150, 200, 250, 300 mM) of chloride salts (NaCl, KCl, $CaCl_2$ and $MgCl_2$) on the seed germination and seedling characteristics in the early growing stage was investigated during the period 2021-2022. The seeds of five introduced varieties grown on alluvial-meadow soil type in the territory of the town Plovdiv, Bulgaria were used. The trials were performed in two replicates of 25 seeds for each variant. Germination of seeds was performed between rolls of filter paper with 20 ml of the respective solutions tested. Different chloride salts and their concentrations caused different effects on the studied germination and seedling characteristics. Rice seeds showed a higher germination rate under salinity stress caused by NaCl and KCl than under stress caused by $CaCl_2$ and $MgCl_2$. Increasing the salt concentration extended the mean germination time for all four salts. The highest value of the relative injury coefficient was recorded when rice seeds were treated with $MgCl_2$ at salt concentrations of 150, 200, 250 and 300 mM, as well as when treated with $CaCl_2$ at a concentration of 300 mM. The seedling height reduction varied widely from 14.10% (at 50 mM NaCl) to 100% (at 250 mM and 300 mM $MgCl_2$, 300 mM $CaCl_2$). In general, the application of increasing concentrations of NaCl and KCl salts from 50 mM to 300 mM caused an osmotic stress on rice seeds, while $CaCl_2$ at 300 mM and $MgCl_2$ at 250-300 mM solutions caused a toxicity effect.

Keywords: rice, chloride salts, seed germination, tolerance

INTRODUCTION

Salinization is one of the major abiotic environmental factors globally limiting plant growth and development [1, 2]. The main causes of salinization are rising groundwater levels with high salt sontent, poor quality of drainage irrigation systems, excessive use of fertilizers, high temperatures coupled with increased evaporation as a result of changing climate [3, 4]. Although it is difficult to determine the exact value, the area of salinized soils is increasing, and this phenomenon is particularly intense in irrigated lands. It is estimated that about 20% of irrigated areas producing one-third of the world's food is affected by salinization [5, 6]. Salinization, on the one hand, causes osmotic stress (a decrease in external water potential) that compromises the plant's ability to take up water and, on the other hand, leads to ionic imbalance, also called ionic stress [7, 8]. Harmful effects of high saltinization can be detected throughout the life cycle from inhibition or delay of seed germination to plant death [9].

In general, saline soils consist of different water-soluble salts and exchangeable Na+ with different effects on seed germination. Different crops show specific germination patterns in saline environments [10, 11].

Rice is a soil salinity-sensitive crop and its sensitivity is variable at different stages of growth and development. It is strongest during germination and up to full rooting of plants [12]. Flowering is the other very sensitive stage of crop development. Salinization during pollination and fertilization leads to sterility [13].

The objective of this study was to determine the effect of different concentrations of chloride salts (NaCl, KCl, $CaCl_2$ and $MgCl_2$) on seed germination and growth parameters of rice.

MATERIALS AND METHODS

The effect of six levels (50, 100, 150, 200, 250, 300 mM) of salinization with chloride salts (NaCl, KCl, $CaCl_2$, and $MgCl_2$) on seed germination and early seedling growth parameters in 5 introduced rice cultivars were investigated in 2021 and 2022. Seeds were collected from a comparative variety of trials established in the region of Plovdiv, Bulgaria on alluvial-meadow soil type. Two replicates of 25 seeds were set for each experimental variant. Germination was carried out in a growth chamber between rolls of filter paper with 20 ml of the respective test solutions at a temperature of 25 ± 1°C, for 14 days. Seeds were counted as germinated when the germ has reached at least 2 mm.

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The number of germinated seeds was registered every day until a consistent number was achieved. The following characteristics were calculated: germination (G, %), germination index (GI), mean germination time (MGT, day) and relative injury rate (RIR). GI and MGT were calculated according to Al-Mudaris [14], while RIR according to Li [15]. On the fourteenth day of the experiment, ten seedlings were randomly selected from each treatment with the chloride salts, for measurement of length of the shoot (ShL, cm) and root (RL, cm). Seed vigor index (VI) was calculated using the equation of Abdul-Baki and Anderson [16]. Seedling height reduction (%) was established according to Islam & Karim [17], while Salt Tolerance (ST) - according to Mujeeb-ur-Rahman *et al.* [18]. Mean data from the two-year experiment were analyzed by using ANOVA and Duncan's multiple range test and SPSS22.0 software was used for statistical data processing.

RESULTS AND DISCUSSION

Salinity influences the seed germination *via* osmotic stress, specific ionic effects and oxidative stress, which is reflected in a decrease in germination rate and an elongated germination time [19]. The *Oryza sativa* seeds responded differently at specific chloride salts concentrations, as shown in Table 1. Maximum germination (between 98.20% and 99.80%) occurred in the non-saline treated seeds (control). Rice seeds showed a higher germination rate under salinity stress caused by NaCl and KCl than under stress caused by $CaCl_2$ and $MgCl_2$. The lowest germination was recorded in seeds treated with 250 mM $CaCl_2$ (16.40%), while at high salinity levels of 300 mM $CaCl_2$, no germinated seeds were recorded. On the other hand, at medium salinity levels in the range of 150 - 200 $MgCl_2$ on the 14th day of seed setting for germination, abnormally germinated seeds characterized by poorly developed shoots and absence of any root system were observed in all tested cultivars, while at high $MgCl_2$ concentrations (250 - 300 mM $MgCl_2$) all seeds were dead (Table 1). Therefore, NaCl and KCl at 50 - 300 mM solutions, $CaCl_2$ at 50 - 250 mM solutions and $MgCl_2$ at 50 - 200 mM solutions caused osmotic stress on rice seeds, while $CaCl_2$ at 300 mM and $MgCl_2$ at 250 - 300 mM solutions caused a toxicity effect.

According to Stefanello *et al.* [20], the accumulation of high salt concentrations in cells can deactivate enzymes, inhibit protein synthesis, and prevent seed germination. Barichello *et al.* [21] stated that excess salts cause cytotoxicity and dehydration of cells, which reduces metabolic activity and synthesis of new tissues in the seeds due to low water availability. On the other hand, Liu *et al.* [22] demonstrated in their study that salinity suppresses rice seed germination by reducing the bioactive gibberellin GA content as a result of increasing the inactivation of bioactive GAs. Furthermore, deficiency of bioactive GA inhibited seed germination by reducing α-amylase activity through down-regulation of α-amylase gene expression. Many authors [6, 22-27] also confirmed that NaCl treatment significantly inhibited seed germination and noted a wide variation in germination and growth parameters among studied rice varieties due to different levels of salt applications. Kalhori *et al.* [6] while investigating the effect of four different salts on seed germination and morphological characteristics of *Oryza sativa* L. also found that NaCl, KCl, $MgCl_2$ and $MgSO_4$ had a negative effect on seed germination. They found that among the four salts, NaCl provided better germination even at the highest salinity level of 250 mM. On the other hand, rice seeds under KCl and $MgCl_2$ germinated less at the highest salinity level, suggesting that the cultivar studied has low tolerance to high salinity levels of these salts.

Salinity had a significant increasing effect on the germination time to a defined level. Increasing the salt concentration extended the mean germination time for all four salts. The highest mean germination time was recorded from seeds treated with 300 mM NaCl (9.63 days), following with 300 mM KCl (8.71 days). Differences from the control variants were statistically proven at $p \leq 0.05$. In the experiments with $CaCl_2$ and $MgCl_2$, the most significant differences to the controls were found at 200 mM $CaCl_2$ and at 100 mM $MgCl_2$ (Table 1). Momayezi *et al.* [28] also reported on prolongation of mean germination time in treatments with over 5 dSm^1 NaCl. Kalhori *et al.* [6] noted that the increase in mean germination time is more pronounced at higher salinity levels of NaCl, KCl, $MgCl_2$ and $MgSO_4$. Higher GI values were recorded for the controls and at the lowest concentration of the four salts, indicating a higher percentage and degree of seed germination in the batch studied. As the salt concentrations increased, the GI values significantly decreased, with the lowest value recorded for the $CaCl_2$ variant at a concentration of 250 mM. At the highest concentration, the GI varied in very close values when salting with NaCl and KCl solutions (251.40 and 249.40, respectively) (Table 1).

Table 1. Effect of different chloride salts and their concentrations on germination (G, %), mean germination time (MGT, day) and germination index (GI) of *Oryza sativa* seeds.

Germination (G, %)				
Salinity	NaCl	KCl	$CaCl_2$	$MgCl_2$
0 mM	99.80cA	99.20cA	98.20dA	98.80bA
50 mM	98.80cB	98.80cB	94.60dA	98.40bB
100 mM	98.40cB	98.20cB	93.00dA	97.40bB
150 mM	95.50cC	97.20cC	87.60dB	0.00aA
200 mM	85.70cC	91.40cC	69.40cB	0.00aA
250 mM	71.50bB	72.20bB	16.40bA	0.00aA
300 mM	38.50aB	35.60aB	0.00aA	0.00aA
Mean	84.03	84.66	65.60	42.09
Mean germination time (MGT, day)				
Salinity	NaCl	KCl	$CaCl_2$	$MgCl_2$
0 mM	2.76aB	2.49aAB	2.06bA	2.07bA
50 mM	3.54abB	3.08aAB	2.77bcA	2.97cAB
100 mM	4.18bcB	4.02bAB	3.45cA	3.71dAB
150 mM	4.76cB	4.66bB	4.35dB	0.00aA
200 mM	6.31dB	5.70cB	5.53fB	0.00aA
250 mM	8.02eB	6.75dB	0.00aA	0.00aA
300 mM	9.63fC	8.71eB	0.00aA	0.00aA
Mean	5.60	5.06	2.76	1.25
Germination index (GI)				
Salinity	NaCl	KCl	$CaCl_2$	$MgCl_2$
0 mM	2.76aB	2.49aAB	2.06bA	2.07bA
50 mM	3.54abB	3.08aAB	2.77bcA	2.97cAB
100 mM	4.18bcB	4.02bAB	3.45cA	3.71dAB
150 mM	4.76cB	4.66bB	4.35dB	0.00aA
200 mM	6.31dB	5.70cB	5.53fB	0.00aA
250 mM	8.02eB	6.75dB	0.00aA	0.00aA
300 mM	9.63fC	8.71eB	0.00aA	0.00aA
Mean	5.60	5.06	2.76	1.25

Means within a column that have different lowercase letters (a-f) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$). Means within a row that have different uppercase letters (A-C) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

Table 2. Effect of different chloride salts and their concentrations on the Relative Injury Rate of *Oryza sativa* seeds.

Relative Injury Rate (RIR)						
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM
NaCl	0.010 a	0.014a	0.043a	0.142a	0.285a	0.615a
KCl	0.006a	0.012a	0.022a	0.080a	0.272a	0.642a
$CaCl_2$	0.038b	0.054b	0.109b	0.296b	0.834b	1.000b
$MgCl_2$	0.004a	0.014a	1.000c	1.000c	1.000b	1.000b
Mean	0.015	0.024	0.294	0.380	0.598	0.814

Means within a column that have different lowercase letters (a-c) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

Decreases in germination index with increasing NaCl salinity levels in rice were also confirmed by Pradheeban *et al*. [23] and Ologundudu *et al*. [29].

The relative injury rates for each salt type with respective salinity levels are presented in Table 2. The highest values of the relative injury coefficients were recorded when rice seeds were treated with $MgCl_2$ at concentrations of 150 - 300 mM salt, as well as when treated with $CaCl_2$ at a concentration of 300 mM. Our results are consistent with those obtained by Kalhori *et al.* [6] who observed the serious injure at 200 mM salt concentration of $MgCl_2$ treatments. At the low concentrations of 50 - 100 mM the greatest damage was found when seeds were treated with a $CaCl_2$ solution. NaCl and KCl salts caused a lower relative degree of damage compared to the other two salts.

The four types of salts had different effect on the growth on *Oryza sativa* seedlings. Shoot, root and total length of rice seedlings decreased with the increase in the salt concentrations in all variants of salinity stress. $CaCl_2$ and $MgCl_2$ salts induced significantly greater negative effect on these variables when compared to NaCl and KCl (Table 3). At the lowest concentration of 50 mM of the studied salts, the greatest ShHR was observed for the $CaCl_2$ variant (36.92%) and in terms of RHR - for the $MgCl_2$ variant (51.50%), but the differences in values compared to the $CaCl_2$ variant were not statistically significant. At the highest concentration of 300 mM the NaCl and KCl salts caused a suppressive effect expressed by a reduction in shoot and root height between 89.90% and 95.25% for shoot and 95.68 - 96.59 % for root, respectively. $CaCl_2$ and $MgCl_2$ salts had a toxic effect on shoot and root growing. On an average, over the whole experiment, the inhibitory effects of the different chloride salts and their concentrations were higher on the root than on the shoot. Kumari *et al.* [30] in his experiment found that under different salt stress (0 - 2.5%) created by a salt mixture of NaCl, $CaCl_2$, and Na_2SO_4 in a 7:2:1 ratio, the rice root growth was more affected than the shoot growth.

The seedling height reduction widely varied from 14.10% (at 50 mM NaCl) to 100% (at 250 mM and 300 mM $MgCl_2$, 300 mM $CaCl_2$) (Table 5). Our findings are in agreement with the results obtained by Kalhori *et al.* [6], who noted that the rice seedling height is less affected by NaCl treatment at 50-250 mM salinity levels as least reduction percentages were recorded at all NaCl concentrations. Reduction of shoot/root lengths with increasing salinity in rice was also reported by other workers [23, 26, 27, 31-34].

Vigor index represents the germination capacity and growing tendency of the seedling. The vigor of *Oryza sativa* seeds was significantly different when treated by four salinities at different concentrations (Table 4). The vigor indices were significantly reduced as salinity increased. In NaCl and KCl salinity, stress indices were higher than in $CaCl_2$ and $MgCl_2$ salinity stress. The highest vigor indices were recorded in 50 mM NaCl and 50 mM KCl, followed by 100 mM NaCl and 100 mM KCl. This result indicated that the capacity of germination and growing tendency of rice seed was higher when germinated in 50-100 mM NaCl and KCl. Diaguna *et al.* [33] stated that the differences in vigor index and germination percentage at salinity of 2000, 4000 and 6000 ppm of NaCl are due to osmotic stress from salinity which inhibits rice germination. The results obtained by Yousof [35] also indicated that $CaCl_2$ salinity levels (9 dS/m) delayed germination, seed and seedling vigor characters compared with normal salinity (0.3 dS/m).

Table 3. Effect of different chloride salts and their concentrations on the shoot, root and seedling height reductions of *Oryza sativa* L. seeds.

Shoot Height Reduction (ShHR, %)						
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM
NaCl	15.17a	38.90b	67.62ab	82.02a	88.09a	95.25b
KCl	19.19a	38.82a	59.71a	80.58a	87.12a	89.99a
$CaCl_2$	36.92b	61.83b	73.59b	84.93a	98.64b	100.00c
$MgCl_2$	21.58a	61.01b	72.44b	84.90a	100.00b	100.00c
Mean	23.22	50.14	68.34	83.11	93.43	96.31
Root Height Reduction (RHR, %)						
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM
NaCl	12.34a	26.34a	52.76a	72.07a	87.11a	95.68a
KCl	13.65a	28.96a	59.79b	84.29b	93.01b	96.59a
$CaCl_2$	49.11b	80.97b	92.72c	96.22c	99.78c	100.00b
$MgCl_2$	51.50b	90.48c	100.00d	100.00d	100.00c	100.00b
Mean	31.65	56.69	76.32	88.15	94.98	98.07
Seedling Height Reduction (SHR, %)						
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM
NaCl	14.10a	31.50a	58.80a	76.20a	87.50a	95.00a
KCl	16.30a	33.10a	60.60a	83.20b	90.70b	94.10a
$CaCl_2$	44.00b	73.00b	84.90b	91.60c	99.40c	100.00b
$MgCl_2$	38.10b	77.00b	87.60b	93.20c	100.00c	100.00b
Mean	28.13	53.65	72.98	86.05	94.40	97.28

Means within a column that have different lowercase letters (a-d) are significantly different from each other (Duncan HSD test, $P \leq 0.05$).

Table 4. Effect of different chloride salts and their concentrations on the vigor index of *Oryza sativa* L. seedser

Vigor index (VI)				
Salinity	NaCl	KCl	$CaCl_2$	$MgCl_2$
0 mM	1786.59fB	1698.91fB	1220.57eA	1249.54dA
50 mM	1514.06eB	1406.98eB	647.49dA	764.11cA
100 mM	1200.13dB	1121.36dB	305.844cA	274.34bA
150 mM	681.99cC	645.04cC	157.15bB	70.03aA
200 mM	364.32bC	263.55bB	69.51aA	31.84aA
250 mM	161.60aC	112.40abB	7.18aA	0.00aA
300 mM	39.73aB	37.61aB	0.00aA	0.00aA
Mean	821.20	755.12	343.96	341.41

Means within a column that have different lowercase letters (a-f) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$). Means within a row that have different uppercase letters (A-C) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

Table 5. The mean salt tolerance indices of rice at germination and early seedling stage

Germination salt tolerance index							
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM	Mean
NaCl	99.00b	98.59b	94.88c	86.61c	71.54b	38.50b	81.5212
KCl	99.60b	99.00b	97.99c	92.15c	72.78b	35.94b	82.9115
$CaCl_2$	96.23a	94.60a	89.06b	70.45b	16.56a	0.00a	61.1497
$MgCl_2$	99.59b	98.76b	0.00a	0.00a	0.00a	0.00a	33.0575
Mean	98.60	97.74	70.48	62.30	40.22	18.61	64.6600
Seedling salt tolerance index							
Salinity	50 mM	100 mM	150 mM	200 mM	250 mM	300 mM	Mean
NaCl	86.27b	68.66b	41.33b	24.28c	12.82c	5.08b	39.74
KCl	83.72b	66.92b	39.44b	16.93b	9.13b	5.86b	37.00
$CaCl_2$	55.96a	27.10a	15.02a	8.40a	0.59a	0.00a	17.84
$MgCl_2$	61.97a	22.91a	12.43a	6.81a	0.00a	0.00a	17.35
Mean	71.98	46.40	27.05	14.10	5.63	2.74	27.98

Means within a column that have different lowercase letters (a-c) are significantly different from each other (Duncan's multiple range test, $P \leq 0.05$).

Analysis of the results in Table 5 showed that at different chloride salts and doses the salt tolerance indices significantly varied. At salinity levels in the range of 50 - 150 mM solutions, rice seeds showed very high germination tolerance toward all salts tested, ranging between 89.06% and 99.59%, except for the treatment with 150 mM $MgCl_2$ where seeds were sensitive to salinity. At 200 - 250 mM solutions, the tolerance of the *Oryza sativa* seeds varied from very high to high for treatment with 200 - 250 mM NaCl, 200 - 250 mM KCl and 200 mM $CaCl_2$.

Medium tolerance to germination was established at 300 mM NaCl and 300 mM KCl. Very low germination salt tolerance was recorded in the variant with 250 mM $CaCl_2$. Rice seeds were sensitive to germination in 200 - 300 mM solutions of $MgCl_2$ and 300 mM $CaCl_2$. Seedling tolerance indices varied from 86.27% to 0%. At the lowest concentration of 50 mM NaCl and 50 mM KCl rice seeds had very high tolerance of seedling growth. High seedling tolerance indices were recorded in the variants with 50 mM $MgCl_2$, 100 mM KCl, 100 mM NaCl. At average salinity doses (150 - 200 mM), the tolerance was between medium for 150 mM NaCl to very low for treatments with 150 mM $CaCl_2$ and $MgCl_2$, 200 mM solutions of KCl, $CaCl_2$ and $MgCl_2$. In the variants with high concentrations (250 - 300 mM) rice seeds showed very low tolerance of seedling growth in all chloride salts, except for the treatments with 250 mM $MgCl_2$ and 300 mM $CaCl_2$ and $MgCl_2$, where it was sensitive.

CONCLUSIONS

The response of rice seeds to salt stress varied depending on the type and the concentration of salt.

The application of increasing concentrations of NaCl and KCl salts from 50 mM to 300 mM caused osmotic stress on rice seeds, resulting in decreased germination and germination index values, extended mean germination time, increased relative injury rate and reduced seedling height.

At concentrations in the range of 250 - 300 mM of $MgCl_2$ and 300 mM of $CaCl_2$, rice seeds did not germinate because of the toxic effect of the salts.

The inhibitory effect of the different chloride salts and of their concentrations was more pronounced on the root than on the shoot.

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