Bulgarian One Health Journal, *2024, Volume 1, 3* 30-39
ISSN 3033-0408, https://doi.org/10.59496/20241PH1 ***Original article***

Effect of calcium chloride concentration on seed germination and young seedling parameters in rice (*Oryza Sativa* L.)

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Summary

The study was conducted with five rice varieties (Osmanchik 97, Gala, Luna, CL 34, Cameo) in order to determine and compare the effect of increased concentrations of $CaCl_2$ on seed germination during the early stages of plant development. Variations include: control (deionized distilled water), 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM $CaCl_2$ concentrations. Germinating energy, germination rate, germination rate coefficient, germination index degree, germination index, mean germination time, sprout and root length, sprouts and roots fresh and dry weight, seed vitality index and salinity tolerance were determined. The data were analyzed with SPSS program. The results show that the application of increasing concentrations of $CaCl_2$ salinity in the order of 50-200 mM leads to a prolongation of the mean germination time of the seeds and has an inhibitory effect on the growth of sprouts and roots, this effect being more pronounced on the roots . The cultivars Osmanchik 97 and Gala are relatively more tolerant to salinization with low and medium concentrations of $CaCl_2$ in terms of seed germination. At salinity levels from 250 mM to 300 mM $CaCl_2$ solution, all tested cultivars were sensitive.

Keywords: *rice, salinity, $CaCl_2$, germination, tolerance*

Introduction

Salinization is one of the most common soil degradation processes (Penkov et al. 2008). Natural or primary salinization is common in arid and semiarid regions of the earth and is caused by the accumulation of high concentrations of salts in soils or groundwater over prolonged geological periods. Secondary salinization occurs when improper agro-remediation is carried out. Nearly half of all irrigated soils worldwide are affected by salinization, alkalinization and waterlogging (Kancheva and Borisova, 2012; Zhao et al. 2020). According to the FAO, the total area of affected regions is more than 833 million hectares, representing about 9% of the world's land surface (FAO, 2021). Soil salinization in Bulgaria covers 35,500 ha of arable land, with about 252 ha of agricultural land salinized by industrial activities (https://www.unccd.int/sites/default/files/naps/2021-04/NAP_2014-2020.pdf).

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Soil salinization is one of the major abiotic constraints to crop production, food security, and adversely affects socioeconomic structure globally (Allakhverdiev, 2000; Kazemi and Eskandari, 2011; Fogliatto et al. 2021). Rice is one of the cereals susceptible to salinization with an EC range of 0 to 8 dS m^{-1} (Fogliatto et al. 2021) and its sensitivity varies during different stages of growth and development (Fogliatto et al. 2021, Makihara et al. 1999; Todaka et al. 2011).

Seed germination is a crucial stage in the life of many plants and resistance to salinity during this period is important for its establishment (Maranon and Gars, 1989; Almansouri et al. 2001). Under physiological conditions, the germination ability of one crop differs from another and even varies among varieties of the same crop (Pradheenban et al. 2014).

Salinity affects seed germination by creating osmotic stress due to reduced water uptake or by ion imbalance due to the toxic effects of sodium (Na+) and chloride (Cl-) ions (Munns and Tester, 2008; Bhusan et al. 2016; Machado and Selarraleiro, 2017; Radanielson et al. 2018; Nagarajan et al. 2022).

The objective of this study was to determine and compare the effect of increasing $CaCl_2$ concentrations on seed germination and during the early stages of plant development of introduced rice cultivars under laboratory conditions.

Material and methods

Five introduced rice varieties (Osmanchik 97, Gala, Luna, CL 34, Cameo) were included in the study. The experiment was conducted in 2022 at the seed testing laboratory of the National Genebank of IPGR-Sadovo. Seeds for treatment were taken from a comparative varietal trial developed in the territory of the town Plovdiv, on alluvial-fallow soil type. From each variety 7 variants were set: control (deionized distilled water), 50 mM, 100 mM, 150 mM, 200 mM, 250 mM and 300 mM $CaCl_2$ solution. Seeds were subjected to dry pre-cooling in a refrigerator for 3 days at 5°C in paper bags.

The experiment was performed in two replicates of 25 seeds for each variant. Germination of seeds was performed between rolls of filter paper (Grade FT 55) with 20 ml of the respective solutions tested. The rolls were placed in sealed polythene bags to avoid moisture loss. The filter paper was changed every two days to prevent salt accumulation.

Seed germination was carried out in the dark in a BINDER growth chamber at 25 ± 1°C for 14 days. Any seed with a germ length of at least 1 mm is considered germinated. Germination energy (GE, %) and germination (G, %) were recorded on days 5 and 14 after seed set. Coefficient of velocity of germination (CVG, % day^{-1}) was calculated according to

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Kader and Jutzi (2004), germination index (GI), germination rate index (GRI) and mean germination time (MGT, day) according to the formula of Kader (Kader, 2005).

Biometric measurements were made on shoot (LSh) and root (LR) length (cm) and fresh and dry weights (mg) of shoot and root (FWSh, FWR, DWSh and DWR) were determined. A total of 20 plants were measured and weighed from each experimental variant, i.e. 10 plants per replicate.

Seed vigour index (VI) was determined using the equation of Florez et al. 2007. Salinity tolerance was calculated using the formula given by Mujeeb-ur-Rahman et al. 2008. The evaluation of the varieties was according to the following scale:

Salinity tolerant index	**Degree of tolerance**
greater than 80%	very high
80-60%	high
60-40%	medium
40-20%	low
less than 20%	very low

The experimental data were processed by analysis of variance (ANOVA) and Duncan's multiple test (Duncan, 1955) using SPSS 22.0 software.

Results and discussion

Increasing salt solution levels from 50 to 200 mM $CaCl_2$ generally had a suppressive effect on germination energy and germination (Table 1). At high salinity levels of 250-300 mM $CaCl_2$ solution, no germinated seeds were recorded in all cultivars tested.

Table 1. Variation in germination characteristics among rice varieties tested at different levels of $CaCl_2$ salinity

Means in the same column followed by the same letters were not significantly different at $p \leq 0.05$, according to Duncan's test.

The application of increasing salt stress had a significant negative effect on coefficient of velocity of germination (CVG, % day^{-1}), germination rate index (GRI) and germination index (GI). The highest CVG, GRI and GI were recorded in the control treatments and with increasing salinity levels these characteristics decreased significantly (Table 1).

At salinity levels of 200 mM $CaCl_2$, demonstrably highest values for CVG were found in Osmanchik 97 (21.98 % day^{-1}) and CL 34 (22.11 % day^{-1}), for GRI and GI, respectively in Gala (15.52 and 736) and Osmanchik 97 (16.94 and 774), while lowest values for CVG, GRI and GI were recorded in Cameo (Table 1).

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Increasing the salinity concentration from 0 to 200 mM $CaCl_2$ increased the mean germination time (MGT, day). At a concentration of 200 mM $CaCl_2$, MGT ranged from 4.55 days (for Osmanchik 97) to 7.85 days (for Cameo) (Table 1).

Shoot and root lengths are the most important indicators in assessing the tolerance of varieties to salinity, since the roots are in direct contact with the soil and absorb water and nutrients from it, and the shoot supplies the rest of the plant.

Table 2. Effect of $CaCl_2$ concentration on seedling performance of rice varieties

Cultivar	Shoot length, cm	Root length, cm	Wet weight of the shoot, mg plant^{-1}	Wet weight of the shoot and root, mg plant^{-1}	Dry weight of the shoot, mg plant^{-1}	Dry weight of the root, mg plant^{-1}	Vigor index
0 mM $CaCl_2$							
Osmanchik 97	4.76a	6.77bc	39.30bc	8.20cd	3.65ab	1.35c	1152.50ab
Cameo	5.42ab	6.09b	45.35c	6.85bc	7.85c	0.75a	1150.50ab
Luna	5.60bc	6.32bc	32.00a	5.20ab	3.10a	0.80a	1168.85ab
Gala	6.14bc	7.03c	43.75c	9.80d	4.00b	1.05b	1316.50b
CL 34	6.22c	5.11a	34.00ab	4.30a	3.60ab	0.80a	1021.35a
Mean	5.63	6.10	38.88	6.87	4.44	0.95	1161.94
50 mM $CaCl_2$							
Osmanchik 97	3.67ab	2.56ab	27.70ab	2.45ab	3.00a	0.45a	598.08bc
Cameo	3.88b	3.90c	37.80d	4.30c	5.35b	0.65a	715.30d
Luna	3.27a	2.8ab	24.80a	2.20a	2.85a	0.40a	545.16ab
Gala	3.50ab	3.11b	31.75bc	3.40bc	3.10a	0.60a	660.50cd
CL 34	3.92b	2.25a	33.75cd	3.30bc	3.15a	0.65a	466.35a
Mean	3.65	2.92	31.16	3.13	3.49	0.55	597.08
100 mM $CaCl_2$							
Osmanchik 97	1.90a	1.02a	19.90a	1.10a	1.95a	0.20a	273.83ab
Cameo	1.96a	1.68b	18.30a	2.40b	2.30a	0.40c	327.01c
Luna	1.82a	0.98a	19.90a	1.00a	1.75a	0.25ab	249.93a
Gala	2.65b	0.97a	26.00b	2.25b	2.65a	0.45c	341.00c
CL 34	3.15c	0.95a	30.35c	1.40ab	3.05a	0.30b	302.42bc
Mean	2.30	1.12	22.89	1.63	2.34	0.32	298.84
150 mM $CaCl_2$							
Osmanchik 97	1.51ab	0.58d	12.55a	1.00b	1.35ab	0.20bc	192.92b
Cameo	1.45ab	0.56cd	10.10a	0.85b	1.10a	0.20bc	132.79a
Luna	1.65b	0.44b	13.35a	0.45a	1.40b	0.10a	183.48ab
Gala	1.26a	0.53c	10.55a	0.90b	2.05c	0.25c	156.53ab

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CL 34	1.96c	0.38a	18.65b	0.45a	1.50b	0.15ab	151.20ab
Mean	1.56	0.50	13.04	0.73	1.48	0.18	163.38
200 mM $CaCl_2$							
Osmanchik 97	1.08b	0.31d	6.35c	0.30a	0.85bc	0.10b	101.26c
Cameo	0.67a	0.19a	2.13a	0.27a	0.37a	0.11b	31.09a
Luna	0.87ab	0.22b	4.24b	0.32a	0.48a	0.11b	54.93b
Gala	0.95b	0.28c	4.90b	0.25a	0.70b	0.10b	88.20c
CL 34	1.09b	0.23b	8.00d	0.30a	0.95c	0.00a	60.54b
Mean	0.93	0.24	5.12	0.29	0.67	0.08	67.20
250 mM $CaCl_2$							
Osmanchik 97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cameo	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luna	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gala	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CL 34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
300 mM $CaCl_2$							
Osmanchik 97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cameo	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luna	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gala	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CL 34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means in the same column followed by the same letters were not significantly different at $p \leq 0.05$, according to Duncan's test.

Analysis of the results showed that increasing salinity levels from 50 to 200 mM $CaCl_2$ solution suppressed shoot and root growth, which was most noticeable at the higher concentrations, and at concentrations of 250-300 mM $CaCl_2$ solution, shoots and roots did not develop (Table 2). The average shoot length varied between 0.67 cm and 6.14 cm. The longest shoot was measured in the control for cultivar CL 34, which, however, was shown to be superior in this parameter only to cultivars Osmanchik 97 and Cameo. Cultivar Cameo is characterized by the shortest germ at 200 mM $CaCl_2$ solution. The average root length varied between 5.11 cm and 7.03 cm in the control and between 0.19 cm (Cameo, 200 mM $CaCl_2$) and 3.90 cm (Cameo, 50 mM $CaCl_2$) at different salinity levels. The greatest decrease in shoot and root length relative to the control, at 200 mM $CaCl_2$ solution, was found in cultivar Gala, by 5.19 cm for the shoot and 6.75 cm for the root, respectively.

Salting had a significant effect on the fresh and dry weight of the shoot and root. Under the influence of the applied increase in salt concentration, the reported values of these

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ISSN 3033-0408, https://doi.org/10.59496/20241PH1 ***Original article***

parameters decreased. At 200 mM $CaCl_2$ solution, the lowest fresh and dry weights per shoot were found in the variety Cameo (2.13 mg plant^{-1} and 0.37 mg plant^{-1}). The least fresh weight per root was recorded for cultivar Gala (0.25 mg plant^{-1}), which was not significantly different from the weights of the other cultivars included in the study at the same stress level. CL 34 was found to have the lowest dry weight per root (0.00).

As the saline concentration increased, a decrease in vigour index values was recorded. The greatest inhibitory effect on the growths was found in variety Cameo, where the vigour index was only 31.09 at 200 mM $CaCl_2$ solution.

Table 3. Salt tolerance indices at different levels of $CaCl_2$ salting in rice varieties

Cultivars	**Levels of salting with**						**Mean**
	50 mM	**100 mM**	**150 mM**	**200 mM**	**250 mM**	**300 mM**	
Germination salt tolerance index							
Osmanchik 97	96.00	94.00	92.00	74.00	0.00	0.00	59.33
Luna	91.84	91.84	89.80	51.02	0.00	0.00	54.08
CL 34	84.44	82.22	71.11	51.11	0.00	0.00	48.15
Cameo	92.00	90.00	66.00	36.00	0.00	0.00	47.33
Gala	100.00	94.00	88.00	72.00	0.00	0.00	59.00
Mean	92.86	90.41	81.38	56.83	0.00	0.00	53.58
Shoot salt tolerance index							
Osmanchik 97	77.18	39.96	31.76	22.61	0.00	0.00	28.58
Luna	58.36	32.53	29.40	15.51	0.00	0.00	22.63
CL 34	62.99	50.60	31.46	17.46	0.00	0.00	27.09
Cameo	71.59	36.16	26.75	12.30	0.00	0.00	24.47
Gala	57.00	43.16	20.44	15.47	0.00	0.00	22.68
Mean	65.42	40.48	27.96	16.67	0.00	0.00	25.09
Root salt tolerance index							
Osmanchik 97	37.81	14.99	8.57	4.58	0.00	0.00	10.99
Luna	44.26	15.44	6.97	3.47	0.00	0.00	11.69
CL 34	43.93	18.49	7.44	4.40	0.00	0.00	12.38
Cameo	64.01	27.53	9.12	3.09	0.00	0.00	17.29
Gala	44.20	13.81	7.47	3.91	0.00	0.00	11.57
Mean	46.84	18.05	7.91	3.89	0.00	0.00	12.78
Seedling salt tolerance index							
Osmanchik 97	54.06	25.29	18.13	12.02	0.00	0.00	18.25
Luna	50.88	23.47	17.51	9.13	0.00	0.00	16.83
CL 34	54.39	36.11	20.62	11.57	0.00	0.00	20.45
Cameo	67.58	31.59	17.43	7.43	0.00	0.00	20.67
Gala	50.17	27.50	13.52	9.30	0.00	0.00	16.75
Mean	55.42	28.79	17.44	9.89	0.00	0.00	18.59

ISSN 3033-0408, https://doi.org/10.59496/20241PH1 ***Original article***

Table 3 shows the salt tolerance indices of the five rice varieties studied. Analysis of the results showed that all the varieties tested were sensitive to the application of high levels of $CaCl_2$ salinity (250-300 mM $CaCl_2$).

At salinity levels in the range of 50-150 mM $CaCl_2$ solution, all varieties showed very high to high germination tolerance ranging between 66% and 100%. At 200 mM $CaCl_2$ solution, the tolerance of the varieties ranged from high for Osmanchik 97 (74%) and Gala (72%), to medium for Luna (51.02%) and SL 34 (51.11%) and to low for Cameo (36%). At the highest $CaCl_2$ concentrations, all tested cultivars were sensitive.

Regarding the tolerance of shoot to salinity, the tested varieties at salinity levels of 50 mM $CaCl_2$ solution were characterized by high and medium tolerance. The index ranged from 57% to 71.59%. Osmanchik 97 and Cameo had high tolerance of shoot to salinity, while the other three varieties had medium tolerance. At salinity levels in the range of 100 mM $CaCl_2$ solution, cultivars CL 34 and Gala showed medium tolerance, while Osmanchik 97, Luna and Cameo, showed low tolerance, respectively. At 150 mM $CaCl_2$ solution, the tolerance of the varieties was low. At salinity levels of 200 mM of $CaCl_2$ solution, all varieties exhibited very low sprout tolerance to salinity except Osmanchik 97 which had low.

At 50 mM $CaCl_2$ solution, the root salt tolerance index ranged from 37.81% to 64.01%. Cameo exhibited high tolerance and Osmanchik 97 exhibited low tolerance. Other varieties included in the study had medium root tolerance to $CaCl_2$ salinity. At levels of 100 mM $CaCl_2$ solution, the tolerance of the cultivars ranged from low for Cameo to very low for the remaining cultivars. Under the influence of the applied medium levels of 150-200 mM $CaCl_2$ solution, all tested cultivars showed very low root tolerance to salinity.

The mean value of the salt tolerance index of the seedling ranged from 7.43% at 200 mM $CaCl_2$ solution to 67.58% at 50 mM $CaCl_2$. At medium salinity levels, except for CL 34, which exhibited low tolerance, all cultivars were characterized by very low seedling tolerance to $CaCl_2$ salinity.

Conclusions

The application of increasing concentrations of $CaCl_2$ salinity in the range of 50-200 mM solution results in a prolongation of the average seed germination time and has an inhibitory effect on shoot and root growth, this effect being more pronounced on the roots.

Seed germination was found to completely stop at salinity levels of 250 mM and 300 mM $CaCl_2$ solution. Relatively the most tolerant to salinity with low and medium $CaCl_2$ concentrations in terms of seed germination were the varieties Osmanchik 97 and Gala.
Osmanchik 97 exhibited high tolerance to salinity in terms of shoot and root growth at 50 mM $CaCl_2$ solution. At salinity levels in the range of 100 mM $CaCl_2$ solution, CL 34 and Gala varieties had medium tolerance in terms of shoot growth, while in terms of root growth the tolerance of the varieties ranged from low to very low.At salinity levels of 150-200 mM of $CaCl_2$ solution, the varieties tested were characterized by low to very low tolerance of their shoot and root growth. The rice varieties tested responded with a decrease in fresh and dry weight per shoot and root to elevated $CaCl_2$ concentrations.

References

Allakhverdiev I, Sakamoto A, Nishiyama Y, Inaba M and Murata N, 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystem I and II in Synechococcus sp., Journal of Plant Physiology, 123 (3): 1047-1056. DOI: 10.1104/pp.123.3.1047

Almansouri M, Kinet J and Lutts S, 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil, 231: 234-254.

Bhusan D, Das D, Hossain M, Murata Y and Anamul Hoque Md, 2016. Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. Australian Journal of Crop Science (AJCS), 10(1): 50-56.

Duncan B, 1955. Multiple range and multiple F tests. Biometrics, 1955: 11, 1-42. DOI: 10.2307/3001478

FAO, 2021. The World Map of Salt Affected Soil, https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/global-map-of-salt-affected-soils/en/

Fogliatto S, Patrucco L, Milan M and Vidotto F, 2021. Sensitivity to salinity at the emergence and seedling stages of barnyardgrass (*Echinochloa crus-galli*), weedy rice (*Oryza sativa*), and rice with different tolerances to ALS-inhibiting herbicides. Weed Science, 69(1): 39-51. DOI:

DOI: 10.1017/wsc.2020.72

Kader A, 2005. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales, 138: 65–75.

Kancheva R and Borisova D, 2012. Remote sensing of saline soils. Yearbook of the Mining and Geological University "Sv. Ivan Rilski", vol. 55: 155-159.

Kazemi K and Eskandari H, 2011. Effects of salt stress on germination and early seedling growth of rice (*Oryza sativa*) cultivars in Iran. African Journal of Biotechnology, 10(77): 17789-17792. DOI: 10.5897/AJB11.2219

Machado A and Selarraleiro R, 2017. Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticultural, 30(2): 30. DOI: 10.3390/horticulturae3020030

Makihara D, Tsuda M, Morita M, Hirai Y and Kuroda T, 1999. Effect of salinity on the growth and development of rice (*Oryza sativa* L.) varieties. Jpn. J. Trop. Agr., 43(4): 285-294. DOI: 10.11248/jsta1957.43.285

Maranon T, Gars A and Trouncoso A, 1989. Salinity and germination of annual Melilotus from the Guadalquivir delta (SW Spain). Plant and Soil, 119: 223-228

Mujeeb-ur-Rahman U, Soomro A, Zahoor-ul-Haq M and Gul Sh, 2008. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. World Journal of Agricultural Sciences, 4 (3): 398-403.

Munns R and Tester M, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651-681. DOI: 10.1146/annurev.arplant.59.032607.092911

Nagarajan S, Varatharajan N and Gandhimeyyan R, 2022. Understanding the responses, mechanism and development of salinity stress tolerant cultivars in rice. Integrative Advances in Rice Research, 91.

Penkov M, Zakharinov B and Peychinova M, 2008. On degradation processes in soils of Bulgaria. Ecology and Future, 7(1):11-17.

Pradheenban L, Nissanka N and Suriyagoda L, 2014. Clustering of Rice (*Oryza sativa* L.) Varieties Cultivated in Jaffna District of Sri Lanka based on Salt Tolerance during Germination and Seedling Stages. Tropical Agricultural Research, 25(3): 358-375.

Radanielson M, Angeles O, Li T, Ismail A and Gaydon D, 2018. Describing the physiological responses of different rice genotypes to salt stress using sigmoid and piecewise linear functions. Field Crops Research, 220: 46–56. DOI: 10.1016/j.fcr.2017.05.001

Zhao Ch, Zhang H, Song Ch, Zhu J and Shabala S, 2020. Mechanisms of Plant Responses and Adaptation to Soil Salinity. In Innovation, 1(1): 1-41. DOI: 10.1016/j.xinn.2020.100017