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Original Contribution

EFFECT OF SALT STRESS ON RICE SEED GERMINATION AND SEEDING GROWTH

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ABSTRACT
PURPOSE: the purpose of the study was to analyse the influence of different NaCl concentrations on germination and seedling parameters in three rice cultivars belong to species *Oriza sativa* L. - Osmanchik 97, Kameo and Luna. METHODS: six NaCl salt concentrations were studied (low concentrations: 25 mM and 50 mM NaCl solutions, medium concentrations: 75 mM and 100 mM NaCl solutions and high concentrations: 125 mM and 150 mM NaCl solutions). Germination data and seedling parameters were recorded. RESULTS: The application of salt concentrations between 25 and 150 had no significant effect on seed germination, while suppressive effect on seedling characteristics. At 150 mM NaCl mean germination time ranged from 2.10 days (for Osmanchik 97) to 3.15 days (for Kameo), mean ShL ranged from 3.65 cm for Kameo to 5.33 cm for Osmanchik 97. With increasing concentrations of NaCl vigor index decreased from 2043 for Osmanchik 97 (control) to 1050.24 for the variety Kameo. CONCLUSION: At 150 mM NaCl concentrations, the studied varieties showed very high salt tolerance to seed germination, high salt tolerance to root growth and medium salt tolerance to shoot growth. The study confirmed that rice cultivars at the stage of seed germination are more tolerant to salinity than at the early seedling stage.

Key words: rice, *Oriza sativa* L., germination, salinity, seedling growth

INTRODUCTION

Rice as the major food source around the world is very sensitive to salt. The sensitivity of rice to stress caused by salinity varies according to the growth phases. The susceptibility of rice to salinity stress varies with growth stages (1-3). Germination phase, early seedling phase and the productive phase are the three most critical phases in the process of evaluation rice for salt stress tolerance (4). Rice varieties at the early growth stage were more sensitive to salinity than at the seed germination stage (1, 5-7). Varieties of the same species have also been described as showing significant variation in tolerance to salinity (8, 9, 10). Plant response to salinity is affected of the magnitude of the specific tolerance and phenotypic plasticity to environmental features (11, 12).

The development of salt tolerant varieties has been regarded among the strategies to increase rice production under saline conditions (10). Kakar et al. (13) noted two important elements for defining genotypes tolerant to salinity: 1) an assessment of salinity sensitive growth stages and 2) seedling characteristics connected with tolerance to salinity stress. Several studies at germination and the early seedling stage have been done using different NaCl concentrations (1, 4, 6, 8, 10, 14-16). Therefore, the purpose of our study was to analyse the influence of different NaCl concentrations on germination and seedling parameters in three rice cultivars belong to species *Oriza sativa* L.

MATERIALS AND METHODS

Three of the most grown in the territory of Bulgaria rice cultivars - Osmanchik 97 (Turkish cultivar), Kameo and Luna (Italian cultivars) were included in the study.

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The experiment was conducted in the Seed Control and Seed Quality of IPGR-Sadovo in 2020. The influence of seven levels of salinity was studied (0 mM, 25 mM, 50 mM, 75 mM, 100 mM, 125 mM and 150 mM NaCl solutions) on the germination and early seedling parameters of studied rice varieties. Before starting the experiment dry cooling of the seeds was carried out by placing them in a refrigerator for three days at 5°C in neutron bags. Two repetitions of 25 seeds per treatment were used to germinate between rolls of filter paper with 20 ml of the respective solutions tested. The paper is changed every two days to remove salt accumulation. The paper rolls were placed in sealed polythene bags to prevent the waste of moisture. Seed germination was performed in a BINDER growth chamber, at a temperature of 25 ± 1°C, for 14 days. Seeds were counted as germinated when the germ has reached at least 2 mm. The number of seeds germinated was registered every day until a consistent number was achieved. From the number of germinated seeds, the following characteristics were calculated: germination energy (GE, %); final germination percentage (G, %), coefficient of velocity of germination (CVG, % day^{-1}), germination rate index (GRI), germination index (GI) and mean time (MGT, day). CVG, GRI, GI, MGT was estimated according to Al-Mudaris (17).

Biometric measurements were made on the length of the shoot (ShL, cm) and root (RL, cm), fresh and dry weight (mg $plant^{-1}$) per shoot and root (ShFW, RFW, ShDW, and RDW) on the on the fourteenth day of the experiment. ShDW, and RDW were determined after oven drying at 80°C for 24 hours. A total of 20 seedlings 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. (18).

Tolerance to salinity was determined according to Mujeeb-ur-Rahman et al. (19). The evaluation of the accessions was on the following scale:

Salt Tolerance Index	Degree of tolerance
> 80%	very high
80-60%	high
60-40%	medium
40-20%	low
< 20%	very low

For statistical and mathematical processing of experimentally obtained data were used analysis of variance (ANOVA), LSD and Duncan's multiple test (20), at levels of statistical significance levels of $p \leq 0.05$. SPSS22.0 software was used for statistical data processing.

RESULTS AND DISCUSSION

In **Figure 1 and Table 1** are presented the influence of applied NaCl solutions on the germination parameters (GE (%), G (%), CVG (% day^{-1}), GRI, GI and MGT (day)) in the tested rice varieties. The studied varieties showed different responses to the tested levels of salinity. In Osmanchik 97 cultivar, increasing the soluble salt levels from 25 to 150 mM NaCl had no suppressive effect on the parameters GE and G, while in Kameo cultivar, the suppressive effect on the same parameters was documented at 100-150 mM NaCl. A statistically significant decrease in germination energy and germination for the Luna variety was found at the highest levels of NaCl **(Figure 1).**

Asch and Wopereis (21) and Sheng et al. (22) reported a negative effect of increasing salt concentrations on seed germination. Rahman et al. (8) established that salinities up to 0.3% NaCl retarded germination but did not decrease final germination. Hakim et al. (1) found that at doses of 20 dS m^{-1} sodium chloride rice seeds did not germinate. Abbas et al. (10) concluded that the different rice cultivars differ in their salt tolerance with respect to seed germination and seedling growth. Munns et al. (23), Flower and Colmer (9), Sarkar et al. (24) noted that salinity affects seed germination process through osmotic stress, ion-specific effects, oxidative stress and hormonal imbalances, shown by decreasing germination rate and extended germination time. In this study a statistically proven negative effect on the CVG, GRI and GI were established, when applying gradual increasing salt concentrations. The highest values of CVG, GRI and GI were recorded at control treatments and with increasing salinity levels the values of these indices decreased.

At the highest NaCl concentration Osmanchik 97 cultivar differed significantly from the other two cultivars from a CVG and GRI. Considerable variations were found between cultivars at levels above 150 mM NaCl for GI **(Table 1).** In the variant with 150 mM solution of NaCl, CVG ranged from 31.82 % day^{-1} (Kameo) to 47.73% day^{-1} (Osmanchik 97), GRI from 32.50 (Kameo) to 49.57 (Osmanchik 97), and GI from 1138 (Kameo) to 1290 (Osmanchik 97). With increasing concentration from 0 to 150 mM NaCl, the MGT increased. At 150 mM NaCl MGT ranged from 2.10 days (for Osmanchik 97) to 3.15 days (for Kameo) **(Table 1).**

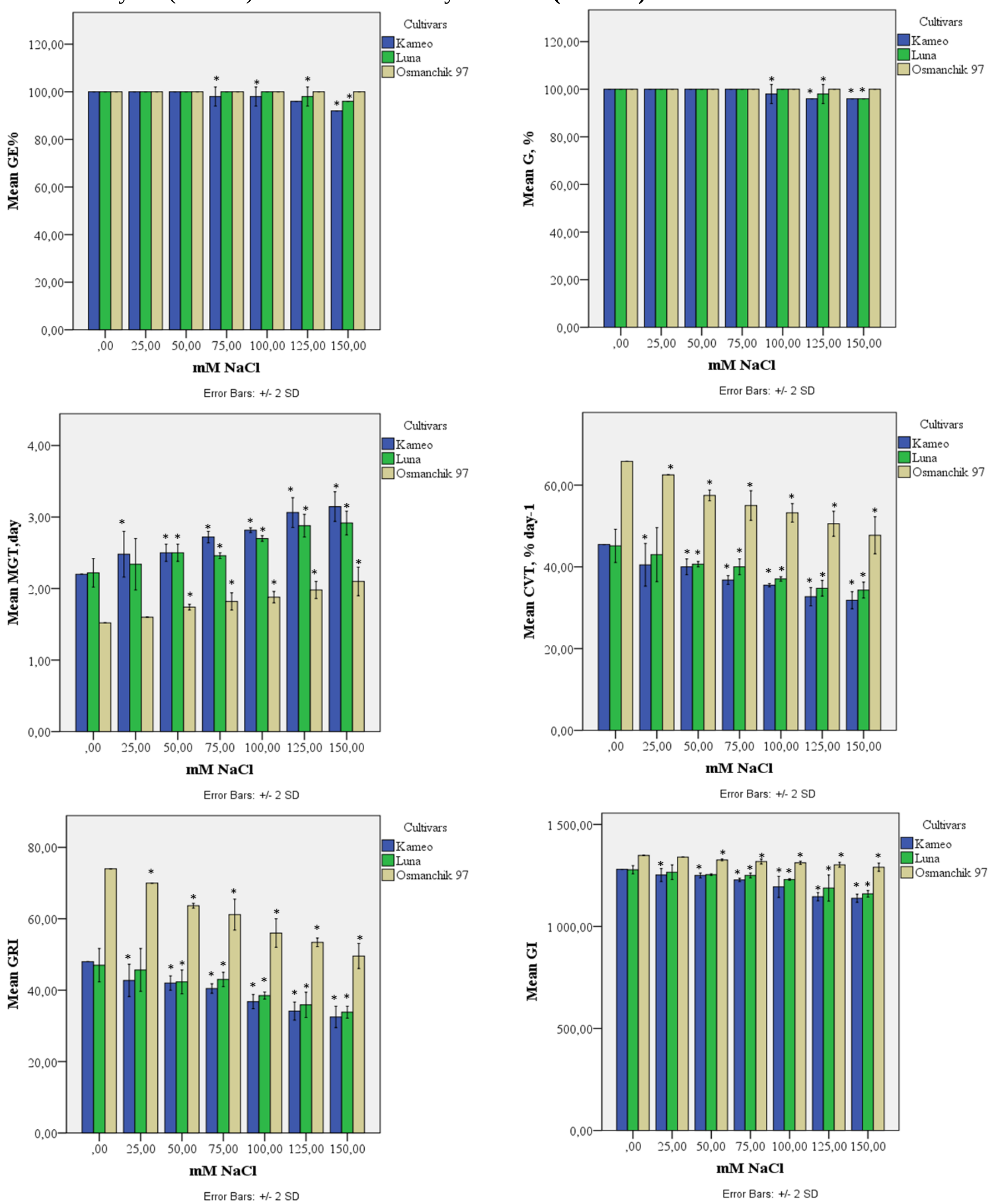

Figure 1. Influence of studied NaCl concentrations on germination energy (GE, %), germination percentage (G, %), mean germination time (MGT, day),coefficient of velocity of germination (CVG, % day^{-1}), germination rate index (GRI) and germination index (GI) for rice cultivars. Data presented are mean ± SD (Standard deviation).
* - statistically proven differences between control variant (0 mM NaCl) and studied salinity levels according to LSD test at statistical levels $p \leq 0.05$.

Table 1. *Influence of the NaCl concentrations on the germination characteristics in tested 5 rice varieties*

Cultivars	**GE%**	**G%**	**MGT,day**	**CVG, % day^{-1}**	**GRI**	**GI**
0 mM NaCl						
Luna	100.00	100.00	2.22b	45.14a	47.00a	1278.00a
Kameo	100.00	100.00	2.20b	45.45a	48.00a	1280.00a
Osmanchik 97	100.00	100.00	1.52a	65.79b	74.00b	1348.00b
Average	100.00	100.00	1.98	52.13	56.33	1302.00
CV, %	**0.00**	**0.00**	**17.61**	**19.76**	**23.62**	**2.68**
25 mM NaCl						
Luna	100.00	100.00	2.34b	42.98a	45.67a	1266.00a
Kameo	100.00	100.00	2.48b	40.49a	42.73a	1252.00a
Osmanchik 97	100.00	100.00	1.60a	62.50b	70.00b	1340.00b
Average	100.00	100.00	2.14	48.66	52.80	1286.00
CV, %	**0.00**	**0.00**	**19.95**	**21.88**	**24.81**	**3.32**
50mM NaCl						
Luna	100.00	100.00	2.50b	40.65a	42.33a	1254.00a
Kameo	100.00	100.00	2.50b	40.02a	42.00a	1250.00a
Osmanchik 97	100.00	100.00	1.74a	57.48b	63.67b	1326.00b
Average	100.00	100.00	2.25	46.05	49.33	1276.67
CV, %	**0.00**	**0.00**	**17.02**	**18.67**	**21.88**	**2.91**
75 mM NaCl						
Luna	100.00a	100.00	2.46b	40.02b	43.00a	1250.00b
Kameo	98.00a	100.00	2.72c	36.77a	40.47a	1228.00a
Osmanchik 97	100.00a	100.00	1.82a	55.00c	61.17b	1318.00c
Average	99.33	100.00	2.33	43.93	48.21	1265.33
CV, %	**1.42**	**0.00**	**17.17**	**19.32**	**20.44**	**3.23**
100 mM NaCl						
Luna	100.00a	100.00a	2.70b	37.04b	38.50a	1230.00b
Kameo	98.00a	98.00a	2.82c	35.50a	36.80a	1194.00a
Osmanchik 97	100.00a	100.00a	1.88a	53.22c	56.00b	1312.00c
Average	99.33	99.33	2.47	41.92	43.77	1245.33
CV, %	**1.42**	**1.42**	**17.96**	**20.32**	**21.19**	**4.34**
125mM NaCl						
Luna	98.00 ab	98.00ab	2.88b	34.76a	35.90a	1188.00b
Kameo	96.00a	96.00a	3.06c	32.69a	34.13a	1146.00a
Osmanchik 97	100.00b	100.00b	1.98a	50.55b	53.40b	1302.00c
Average	98.00	98.00	2.64	39.33	41.14	1212.00
CV, %	**2.04**	**2.04**	**19.2**	**21.68**	**22.58**	**5.94**
150 mM NaCl						
Luna	96.00ab	96.00a	2.92b	34.31a	33.83a	1160.00b
Kameo	92.00a	96.00a	3.15c	31.82a	32.50a	1138.00a
Osmanchik 97	100.00b	100.00b	2.10a	47.73b	49.57b	1290.00c
Average	96.00	97.33	2.72	37.95	38.63	1196.00
CV, %	**3.61**	**2.05**	**17.76**	**19.84**	**21.52**	**5.99**

Germination energy (GE, %), germination percentage (G, %), mean germination time (MGT, day), coefficient of velocity of germination (CVG, % day-1), germination rate index (GRI) and germination index (GI), coefficient of variation (CV, %)
Subscripts (a-c) with different letters in the column indicated significant difference among mean ($p \leq 0.05$,using Duncan multiple range test)

The negative effect of salinization can also be expressed by the change in the morphological characteristics of plants. In the **Table 2** are presented the influence of NaCl concentrations on the ShL and RL in tested rice varieties. Increasing the level of NaCl in the solutions showed suppressive effect on ShL and RL and on their corresponding fresh and dry **weight (Table 2 and Table 3).** At 150 mM NaCl, the mean ShL ranged from 3.65 cm for Kameo to 5.33 cm for Osmanchik 97. In Luna cultivar was recorded, respectively, the largest average root length of 10.66 cm in the control and the smallest of 6.99 cm in the 150 mM NaCl. The longest root length was recorded for Osmanchik 97 at the highest concentration, but differences with respect to the other varieties in the experiment were not statistically proven at a statistical significance level of $p \leq 0.05$ **(Table 2).**

Mean seedling length ranged from 10.94 cm to 18.64 cm. Osmanchik 97 had the longest seedling length at all salt levels, but it was differ significantly only from Kameo at 0-50 mM and 125-150 mM NaCl **(Table 3).**

There was a positive relationship between vigour index and increasing salinity, showing the inhibitory action of salt stress on seed germination and seedling growth. Significant differences between NaCl treatments and the vigor index, both between the different variants in the experiment and between the studied rice varieties were recorded. With increasing concentrations of NaCl index decreased from 2043 for Osmanchik 97 (control) to 1050.24 for the variety Kameo **(Table 3).**

Table 2. *Impact of NaCl concentrations on the shoot and root length in tested rice varieties*

mM NaCl	Kameo	Luna	Osmanchik 97	Kameo	Luna	Osmanchik 97
	ShL, cm			RL, cm		
0	7.54cA	7.98dA	10.08eB	9.26cA	10.66dA	10.35bA
25	6.00bA	7.64cdB	8.68dC	8.90bcA	8.86cA	9.18abA
50	5.65bA	7.40cdB	6.96cB	8.71abcB	7.99bA	9.04abB
75	5.58bA	7.00cB	6.37bcAB	8.57abcA	7.93bA	8.74abA
100	5.33bA	6.01bA	6.18abcA	8.18abcA	7.62abA	8.52abA
125	5.26abA	5.99bB	6.03abB	7.43abA	7.51abA	8.02aA
150	3.65aA	4.67aB	5.33aB	7.29aA	6.99aA	7.51aA
Mean	5.57	6.67	7.09	8.34	8.22	8.77

Shoot length (ShL, cm), root length (RL, cm)
Subscripts (a-d) with different letters in the column indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)
Subscripts (A-C) with different letters in the row indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)

Table 3. *Influence of NaCl concentrations on the seedling length and vigor index in the tested rice varieties*

mM NaCl	Kameo	Luna	Osmanchik 97	Kameo	Luna	Osmanchik 97
	SL, cm			VI		
0	16.81dA	18.64fAB	20.43eB	1680.50dA	1863.50fAB	2043.00eB
25	14.91cA	16.50eB	17.87dB	1491.00cA	1650.00eB	1786.50dB
50	14.36bcA	15.39deAB	16.01cB	1436.50cA	1538.50deAB	1600.50cB
75	14.15bcA	14.94cdA	15.12bcA	1415.00cA	1493.50cdA	1512.00bcA
100	13.51bcA	13.63bcA	14.71bcA	1325.64bcA	1363.00bcA	1470.50bcA
125	12.69bA	13.50bB	14.05abB	1218.72abA	1322.96bB	1405.00abC
150	10.94aA	11.66aA	12.83aA	1050.24aA	1119.36aAB	1283.50aB
Mean	13.91	14.89	15.86	1373.94	1478.69	1585.86

Seedling length (SL, cm), vigor index (VI)
Subscripts (a-f) with different letters in the column indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)
Subscripts (A-C) with different letters in the row indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)

The application of elevated levels of NaCl had an adverse effect on the parameters - ShFW, RFW, ShDW, and RDW **(Table 4 and Table 5).** No significant inter-genotypic variations were recorded in the salt concentrations tested with respect to the indicator fresh shoot weight. In cultivar Osmanchik 97, at 150 mM NaCl solution, the highest fresh root weight was recorded, but compared to the other two cultivars; differences were not statistically proven with those of cultivar Kameo **(Table 4).**

Kameo was characterized by the lowest dry shoot weight and Luna with the lowest dry root weight at 150 mM NaCl, respectively 3.90 mg plant^{-1} and 1.40 mg plant^{-1}. At 150 mM sodium chloride dilution, cultivar Osmanchik 97 showed statistically proven superiority over cultivar Luna in root dry weight, while no statistical differences were found between the tested cultivars in terms of ShDW **(Table 5).**

Our data confirm the results of studies by Hakim et al. (1), Chang et al. (3), Rahman et al. (8), Kakar et al. (13), Razzaque et al. (14), Jiang (15), Hussain et al. (16), Folkard and Wopereis (25), Fogliatto et al. (26), that elevated salinity levels lead to a reduce ShL and RL, as well as ShFW, RFW, ShDW, and RDW, and the extent of the decline increases with increasing salt stress.

Table 4. *Influence of NaCl concentrations on the fresh shoot weight and fresh root weight in tested rice varieties*

mM NaCl	Kameo	Luna	Osmanchik 97	Kameo	Luna	Osmanchik 97
	FWSH, mg plant^{-1}			FWR, mg plant^{-1}		
0	55.75dA	53.80dA	58.05dA	21.25bA	20.85dA	23.10bA
25	50.80cdA	51.95dA	53.25cA	19.10abA	16.30cAB	21.80bB
50	48.70bcdA	47.80cA	52.85cA	18.75abA	15.50bcA	20.25abA
75	47.95bcA	46.55cA	47.15bA	18.55abB	15.00bcA	19.95abB
100	41.80bcA	44.85bcA	46.80bA	18.20abB	14.65bA	18.80abC
125	45.10abA	41.75abA	45.05bA	16.20aB	13.05aA	18.45abB
150	37.20aA	39.50aA	38.70aA	15.05aAB	12.70aA	16.35aB
Mean	46.7571	46.6000	48.8357	18.1571	15.4357	19.8143

Fresh shoot weight (FWSh, mg plant^{-1}) and fresh root weight (FWR, mg plant^{-1})
Subscripts (a-d) with different letters in the column indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)
Subscripts (A-C) with different letters in the row indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)

Table 5. *Influence of the NaCl concentrations on the dry shoot weight and dry root weight in tested rice varieties*

mM NaCl	Kameo	Luna	Osmanchik 97	Kameo	Luna	Osmanchik 97
	DWSH, mg plant^{-1}			DWR, mg plant^{-1}		
0	6.70eA	6.75dA	7.25dA	3.25dA	3.60dB	4.20cC
25	6.20deA	6.80dAB	7.15dB	2.85cA	3.40dB	3.65bB
50	5.75cdA	6.55dB	6.80dB	2.60bcA	2.50cA	3.55bB
75	5.65cdA	5.60cA	6.70cdB	2.50bA	2.40cA	3.40bB
100	5.0bcA	5.10bcA	6.15bcB	2.40bA	2.00bA	3.25bB
125	4.40abA	4.95bB	5.80bC	2.00aAB	1.90bA	2.20aB
150	3.90aA	4.40aA	4.95aA	1.70aAB	1.40aA	1.90aB
Mean	5.37	5.74	6.40	2.47	2.46	3.16

Dry shoot weight (DWSh, mg plant^{-1}) and dry root weight (DWR, mg plant^{-1})
Subscripts (a-e) with different letters in the column indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)
Subscripts (A-C) with different letters in the row indicated significant difference among mean ($p \leq 0.05$, using Duncan multiple range test)

The response of plants and their ability to tolerate salinity stress varies significantly among species (12). **Table 6** shows the salinity tolerance indices at the different salt concentration for the studied rice varieties. At salinity levels in the range of 25-150 mM of NaCl all varieties showed very high tolerance to seed germination ranging between 96% and 100%. For Luna variety, the shoot tolerant index varied from 58.52 % (at 150 mM NaCl) to 95.74 % (at 25 mM NaCl). At levels in the range of 25-125 mM of NaCl solution, Kameo demonstrated high tolerance to shoot growth, while at 150 mM NaCl medium tolerance. The tolerance of Osmanchik 97 to shoot growth varied from very high (at 25 mM NaCl) to medium (at 125-150 mM NaCl). Luna variety had very high tolerance to root growth at 25 mM NaCl, Kameo respectively between 25 and 125 mM NaCl, while Osmanchik 97 at 25-100 mM NaCl. Root salt tolerant index was high at salinity stress from 50 to 150 mM NaCl for Luna, for Osmanchik 97 from 100 to 150 mM NaCl, and for Kameo variety only at 150 mM NaCl. In general, the studied rice cultivars at the stage of seed germination were more tolerant to salinity than at the early seedling stage. The roots showed greater tolerance to the tested salinity levels than the shoots.

Table 6. *The salinity tolerance indices at different salt concentration for the three rice varieties.*

mM Na Cl	Luna	Kameo	Osmanchik 97	Luna	Kameo	Osmanchik 97
	STIG, %			STISh, %		
25	100.00c	100.00c	100.00	95.74cB	79.59cA	86.11dA
50	100.00c	100.00c	100.00	92.73cC	74.88bcB	69.05cA
75	100.00c	100.00c	100.00	87.78cB	73.96bcA	63.24bcA
100	100.00cA	98.00bA	100.00A	75.31bB	70.64bAB	61.31abcA
125	98.00bAB	96.00aA	100.00B	75.06bB	69.78bB	59.77abA
150	96.00aA	96.00aA	100B	58.52aA	48.31aA	52.83aA
Mean	**99.00**	**98.33**	**100.00**	**80.86**	**69.53**	**65.38**
	STIR, %			STIS, %		
25	83.15cA	96.16aA	88.74aA	88.54cA	88.72cA	87.44dA
50	74.94bcA	94.11aC	87.39aB	82.56cAB	85.48bcB	78.34cA
75	74.43abcA	92.54aB	84.49aAB	80.14bcA	84.20bcA	74.01bcA
100	71.52abA	88.33aA	82.37aA	73.14bA	80.39bcA	71.98bA
125	70.48abA	80.23aA	77.54aA	72.44bAB	75.54bB	68.77bA
150	65.60aA	78.78aA	72.56aA	62.57aA	65.10aA	62.82aA
Mean	**73.35**	**88.36**	**82.18**	**76.57**	**79.91**	**73.89**

Germination salt tolerant index (STIG, %), shoot salt tolerant index (STISh, %), root salt tolerant index (STIR), seedling salt tolerant index (STIS, %)

Subscripts (a-c) with different letters in the column indicated significant difference among mean (p≤0.05, using Duncan multiple range test)

Subscripts (A-B) with different letters in the row indicated significant difference among mean (p≤0.05, using Duncan multiple range test)

CONCLUSION

A negative relationship was found between increasing levels of salt stress and all examined germination and seedling parameters except for the mean germination time. Salinity suppressed shoot more than root development. At 150 mM NaCl concentrations, the studied rice varieties showed very high salt tolerance to seed germination, high salt tolerance to root growth and medium salt tolerance to shoot growth. The study confirmed that rice cultivars at the stage of seed germination are more tolerant to salinity than at the early seedling stage.

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