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Morphophysiological Responses of Rice (*Oryza sativa* L.) Genotypes to Salinity Stress during Germination Stage

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Abstract: Salinity hinders seed germination and causes a delay in emergence, diminishes photosynthesis, promotes senescence, and eventually reduces crop yields. This study examined the impact of soil salinity levels (0, 100, and 200 mM) on seed germination and seedling growth in seven rice cultivars. The parameters, *viz*. germination rate, mean germination time, root and shoot length, root and shoot fresh weight, and seedling height stress index, assessed during the germination and early seedling phases were found to be influenced by salinity, genotypes, and their interactions. Results showed that increased salinity levels led to an increase in germination time and a decrease in germination index. According to the study's findings, cultivars MR211, MR220, and Pokkali had the highest values for the characteristics being examined. In contrast, all cultivars showed a decline in these characteristics as saline levels rose. Under conditions of salt stress, genotypes MR211, MR220, and Pokkhali outperformed other genotypes in terms of seedling growth in root and shoot length. Based on their germination index and average germination time, Pokkhali and BR29 can be categorised as salt-tolerant and salt-sensitive, respectively.

Keywords: Seed germination, Salinity, Seedling growth, Morphophysiological response

Several environmental factors act as inhibitors of plant growth, with salt emerging as a prominent limitation (Ashraf 2004, Munns et al*.,* 2006). Soil salinity is one of the most detrimental effects of climate change on coastal agricultural land, as rising sea levels have increased salinity from 1 to 33% over the last 25 years (Rahman et al*.,* 2018). Plants exhibit heightened vulnerability to environmental stresses during seed germination and early seedling growth (Ashrafuzzaman et al*.,* 2003). A negative correlation between seed germination and increasing salinity levels has been well demonstrated in previous studies. This decline in germination can occur through two mechanisms: osmotically, by reducing water absorption, or ionically, by promoting the accumulation of sodium (Na^+) and chloride (Cl^-) ions. Consequently, this imbalance in nutrient uptake and the presence of toxic ions can hinder the germination process (Sarwar et al., 2020, Tobe et al., 2002). Salinity inhibits the process of seed germination, retards the plant growth, and diminishes the overall yield of crops.

Cereal crops e.g., wheat, maize, rice, and barley, are classified as glycophytes (salt-sensitive), however, salt tolerance can differ both between species and within a species (Shelden and Munns 2023). Rice (*Oryza sativa* L.) holds significant global importance, serving as a dietary staple for more than half of the world's population. There is considerable interest in developing rice cultivars that are both high-yielding and resistant to salinity. Pokkali and Nonabokra are two rice cultivars known for their salt tolerance; nonetheless, production potential is somewhat lower when compared to contemporary rice types. Malaysian Agricultural Research and Development Institute, like other rice-growing countries, has released several high-yielding rice cultivars, including MR84, MR211, MR219, MR220, and MR232. However, the salinity tolerance of these rice cultivars has not yet been verified.

Many studies have been conducted to elucidate rice plants' physiological responses to NaCl salinity stress (Liu et al., 2018). Rice cultivars are salt tolerant during germination, though salinity retards germination, but crop yield is affected due to its increased sensitivity to salinity during early seedling growth. Significant variation in seed germination occurs between rice cultivars grown in salinity conditions, (Liu et al., 2018) extensively. The objective of this study was to investigate the physiology of salt tolerance in rice plants, to assess the tolerance level of Malaysian high-yielding rice cultivars to salinity during germination and early seedling stage in the laboratory before field trials, and to identify cultivars with a high potential for breeding salt-tolerant lines.

MATERIAL AND METHODS

Laboratory experiment was conducted to study germination and seedling growth of different rice cultivars under different salinity levels. Seven rice cultivars namely MR84, MR211, MR219, MR220, MR232, Pokkali, and BR29, were used in this study. The seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. The surface sterilized seeds were placed on Whatman no.1 filter paper lined in the Petri dishes. Twenty-five seeds were kept at equidistance in each Petri dish (9 cm diameter). The filter papers were moistened with saline solutions of 100 and 200 mM. Distilled water-moistened Petri dishes served as control. Petri dishes were sealed with Parafilm and placed on the laboratory bench at room temperature 24-28°C with a 12-hour photoperiod for germination up to ten days. Each treatment was replicated three times in a completely randomized experimental design.

Germination was observed daily according to the recommendation of the International Seed Testing Association (ISTA 1993). Seeds exhibiting radicle emergence (>2 mm) were recorded every day until germination ceased. The total number of seed germinated were counted and the percentage was calculated. The germination index (GI) was calculated after final germination with the following equation:

$$GI = \frac{\text{Germination \% in each treatment}}{\text{Germination \% in the control}} \times 100$$

The mean germination time (MGT) was calculated according to the equation proposed by Ellis and Roberts (1981), as follows: $MGT = \frac{\sum Dn}{\sum n}$

Where MGT is the mean germination time, n is the number of seeds, which were germinated on day D; D is the number of days counted from the beginning of germination.

The rate of germination was estimated using a modified Timson index of germination velocity =Σ G/t, where G is the percentage of seed germination at one-day intervals and t is the total germination period (Khan and Ungar 1984). The maximum possible value using this index with our data was 50 (i.e. 1000/20). The higher the value, the more rapid the rate of germination.

On the 10th day, fresh weight, root, and shoot length of germinated seedlings were measured. Subsequently, the seedlings were placed in an oven at 70°C for 48 hours and dry weights were determined. Seedling fresh and dry weight was computed by adding the fresh and dry weights of root and shoot.

The seedling height is considered the sum of the length of the shoot and root; the seedling height stress index (SHSI) was calculated by the following formula:

$$SHSI = \frac{\text{Seedling height of stressed seedlings}}{\text{Seedling height of control seedling}} \times 100$$

Seed vigor index (SVI) was calculated as:

$$SVI = \frac{\text{Seedling dry weight (SDW)}}{\text{Mean germination time (MGT)}}$$

Water absorption rate (WAR) was calculated using the following formula WAR (%) = 100 (a-b)/b, where a is the weight (g) of seeds after soaking in distilled water for 24 h and b is the initial weight (g) of seed sample.

Proline estimation: Proline content from shoots was extracted according to the method of Bates et al. (1973).

Determination of soluble sugars: Total soluble sugars were determined by the phenol sulfuric acid method (Dubois et al., 1956) using glucose as standard.

Statistical analysis: Statistical analysis was performed using SAS program.

RESULTS AND DISCUSSION

Germination Percent (FGP), Germination Index (GI) and Germination rate: In the control group, all cultivars exhibited 100% final germination. Up to a sodium chloride (NaCl) concentration of 100 mM, the final germination percentage remained consistent across all cultivars. However, notable differences emerged at extreme NaCl concentrations. The germination rates varied among cultivars at specific salt concentrations, with MR84, MR211, MR232, and Pokkali demonstrating the highest germination capability even at 100 mM NaCl. At 200 mM NaCl, there was a significant reduction in the final germination percentage for all cultivars. As NaCl levels increased, the germination index decreased, with a strong negative correlation coefficient between NaCl concentrations and the germination index. High-yielding cultivars demonstrated relatively consistent germination performance. Decline in gibberellic acid (GA) content in seeds due to salinity may contribute to reduced germination rates (Liu et al., 2018).

Mean Germination Time (MGT): In contrast, under normal conditions (control), germination started promptly with minimal variation in germination time. However, salinity caused delays in initiation and decreased the rate of germination, effects that became more pronounced with lower seed water content. The mean germination time (MGT) varied significantly among different treatments, gradually increasing with higher salt concentrations. Among Malaysian HYVs, MR84 exhibited the shortest MGT, statistically similar to other varieties. The escalating salt levels led to a decrease in MGT, indicating a slowdown in germination likely due to inhibited activation of enzymes crucial for reserve hydrolysis and mobilization. This pattern was consistent even at 100 mM. All cultivars managed to germinate under varying NaCl concentrations, the time taken differed based on cultivar and salt concentration. Higher NaCl levels primarily prolonged germination time rather than affecting the final germination percentage. Hence, MGT and germination index could serve as reliable indicators for assessing salt tolerance during germination, as salt-tolerant genotypes typically exhibit the shortest MGT and highest GI.

Water uptake (%): There was a clear relationship between water uptake and salinity, indicating that as salinity levels increase, water uptake decreases. Specifically, the increase in salinity stress significantly reduces water uptake. MR211 exhibits the highest water uptake, while Pokkali shows the lowest (Fig. 1). Variability in seed water uptake among rice cultivars is considerable, ranging from 22.14% to 33.33%, with significant differences. Moreover, water uptake is influenced by seed size. Despite differences in cultivars, their responses to salinity stress vary. Although there's no significant difference in water uptake as NaCl levels rise, seeds absorb water rapidly in the initial 6 hours. Throughout the measurement period, water uptake remains relatively consistent across NaCl levels. Imbibition, or water uptake, marks the initial stage of seed germination, wherein dry seeds, typically containing less than 10% water, absorb water and swell. This process kickstarts essential metabolic activities, including the breakdown of stored starches into sugars for energy and cellular structure formation.

Root length: MR219 showed the maximum root length under non-saline conditions, whereas MR232 had the minimum (Table 2). However, this trend shifted at 100 mM and 200 mM NaCl concentrations. At 100 mM, MR84 displayed the longest root length (34.67 mm), accounting for 72.73% of the control, whereas MR232 and Pokkhali had the shortest lengths. Root length of MR219, MR232, and MR220 was more affected compared to MR211, BR29, and Pokkali at higher salt concentrations indicating that the salt stress not only affected germination but also the growth of seedlings, which indicates that synthetic ability of seed and thus dry matter production of the seedlings was affected. This conforms with the findings of Vibhuti et al. (2015) where they observed that root length was conspicuously affected by salt.

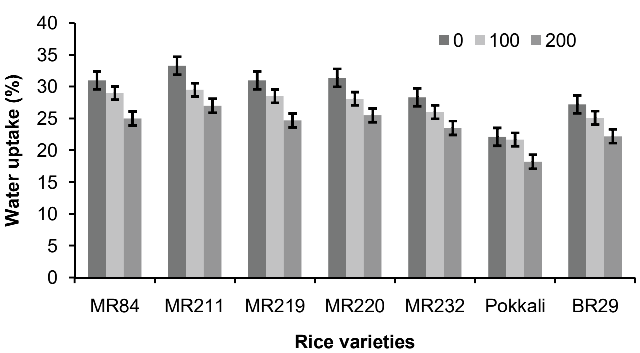

Fig. 1. Effect of NaCl on water uptake (%) of seeds of seven rice cultivars after placing in NaCl treatment at 0, 100 and 200 mM. Bars indicate standard errors

Shoot length: The longest shoot length was observed in Pokkali, followed by MR219, which was statistically similar to other high-yielding Malaysian cultivars, while MR211 exhibited the minimum shoot length under normal conditions. Generally, shoot length decreased gradually as the NaCl concentration increased across all rice cultivars. At 200 mM NaCl, MR84 recorded the longest shoot length (19.18 mm), which accounted for 23.88% of the control. The decline in shoot length due to high salinity was minimal (34.64%) in MR211, while MR219 and MR220 showed the maximum decrease. This indicates that the shoot growth of MR220 was the most affected by NaCl levels.

Seedling height: Pokkali consistently produced the tallest seedlings, maintaining its superiority even in the presence of 100 mM NaCl. However, at 200 mM NaCl, there was a significant reduction in seedling height, down to 22% of the control level (Table 2). Except for MR211, there were no statistically significant differences in seedling height among the rice cultivars. MR211 produced the tallest seedlings at 200 mM NaCl, approximately 50% of the control level, followed by MR84 (Table 2). Both root and shoot lengths experienced substantial decreases with higher salinity levels. Root growth was more severely affected, decreasing

Table 1. Germination percentage, germination index, mean germination time and mean germination rate of some rice cultivars under different salinity levels

Cultivars	Germination (%)			Germination Index		Mean germination time			Mean germination rate		
	0 mM	100 mM	200 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	100.0a	100.0a	93.0ab	100.00a	93.00 ab	6.05d	6.44d	8.18b	37.53a	29.74ab	10.06bc
MR211	100.0a	100.0a	93.0ab	100.00a	93.00 ab	6.13d	6.52d	7.95b	35.68a-c	28.50ab	11.96ab
MR219	100.0a	95.0a	78.0abc	95.00a	78.00 bc	6.07d	6.50d	7.92b	36.98ab	27.08ab	9.88bc
MR220	100.0a	93.0a	87.0ab	93.00a	87.00 a-c	6.10d	6.53d	7.76b	36.32a-c	26.26b	12.46a
MR232	100.0a	100.0a	87.0ab	100.00a	87.00 a-c	6.26c	6.44d	7.97b	33.08c	30.04a	10.95ab
Pokkali	100.0a	98.0a	94.0a	98.00a	94.00 ab	6.17d	6.60c	8.31b	34.98a-c	26.53ab	8.11cd
BR29	100.0a	90.0a	73.0bc	90.00a	73.00 c	6.24d	6.86bc	8.23b	33.66bc	21.29c	6.98de
CV (%)	5.5	6.3	9.5	7.15	7.05	9.5	7.5	2.55	4.76	4.97	8.76

by up to 99% compared to a maximum reduction of 86% in shoot growth (Table 2). Conversely, in some species, root growth may remain unaffected or even increase under low salinity conditions, while shoot growth declines (Shelden and Munns 2023).

Seedling height stress index (SHSI): High salt levels consistently inhibited the growth of rice seedlings across different genetic varieties, as shown by the SHSI. The varieties MR84, MR211, and Pokkali demonstrated better growth at salt concentrations of 0-100 mM, while other varieties showed less favorable results. Some cultivars displayed mixed responses under salt concentrations of 100-200 mM.

Seedling fresh weight: The fresh weight of seedlings was consistently and significantly reduced in all rice cultivars at concentrations between 100 mM and 200 mM (Table 3). The shoot fresh weight of each cultivar fluctuated with varying NaCl concentrations. Seedling fresh weight varied from 38.33 to 92.67 mg. Among the rice cultivars, Pokkali had the highest seedling fresh weight, followed by MR220, which was statistically similar to MR84, MR219, and MR211.

Timson index of germination velocity: Salinity gradually reduced the germination velocity (Timson index). Values of the Timson index did not differ significantly among the rice genotypes in the control (Table 3). At 100 mM NaCl, the highest Timson index was recorded in MR232 which is comparable to MR84 and MR211 while the minimum was recorded in BR29. However, this trend was different at 200 mM NaCl where MR220 possessed the highest value.

Seed vigour index (SVI): Seed vigour index differed significantly among the rice genotypes even in the control environment (Table 3). Rice genotypes responded differently to different salt levels. The SVI sharply decreased as the NaCl levels increased and it was maximum in Pokkali followed by MR220 which was statistically similar to MR84

Table 2. Effect of different NaCl levels on shoot and root length, seedling height and seedling height stress index of rice cultivars.

Cultivars	Shoot length (mm)			Root length (mm)			Seedling height (mm)			SHTI	
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	100 mM	200 mM
MR84	80.33b (100)	22.31c-e (27.78)	19.18a (23.88)	47.67c (100)	34.67a (72.73)	0.67cd (1.40)	128.00b	56.98ab	19.85b	44.52a	15.50bc
MR211	52.00c (100)	25.29c (48.63)	18.01ab (34.64)	45.33cd (100)	16.00c (35.29)	2.68a (5.90)	97.33d	34.01c	27.96a	35.15bc	29.08a
MR219	84.67b (100)	20.98cd (24.78)	13.39d (15.82)	66.00a (100)	14.33cd (21.72)	0.50cd (0.76)	150.67a	35.31c	13.89cd	23.51d	9.27d
MR220	84.33b (100)	33.02b (39.16)	13.19d (15.64)	41.00d (100)	21.00b (51.22)	0.83c (2.03)	125.33b	54.02b	14.02cd	43.31ab	11.18d
MR232	80.67b (100)	21.00cd (26.03)	17.50ab (21.70)	28.33f (100)	12.33cd (43.53)	0.50cd (1.76)	109.00c	33.33c	18.00bc	30.58cd	16.54b
Pokkali	103.67a (100)	56.46a (54.46)	17.26a-c (16.65)	56.33b (100)	12.02cd (21.34)	2.17b (3.85)	160.00a	68.48a	19.43b	42.92ab	12.13cd
BR29	72.33b (100)	25.00c (34.56)	16.00b-d (22.12)	38.33e (100)	15.09c (39.37)	2.00b (5.22)	110.67c	40.09c	18.00bc	36.20a-c	16.27bc
CV (%)	7.2	9.42	7.18	8.0	10.0	9.0	3.2	9.9	9.5	9.4	9.8

SHTI- Seedling height stress index; Values in parentheses represent the percentage relative to untreated control plants (set at 100%)

Table 3. Seedling fresh weight, Timson index of germination velocity and Seed vigor index of rice cultivars

Cultivars	Seedling fresh weight (mg)			Timson index of germination velocity			Seed vigor index (SVI)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	62.00b	40.67b	21.60bc	49.17	41.93	22.47	2.31b	0.88ab	0.45bcd
MR211	52.33c	26.00de	26.03a-c	47.33	41.37	25.37	2.09bc	0.65cd	0.34de
MR219	64.67b	30.97de	24.67a-c	48.73	38.83	20.13	1.86c	0.77bc	0.39cde
MR220	65.00b	39.50bc	20.83cd	48.17	37.37	26.03	2.31b	0.80bc	0.52b
MR232	52.33c	27.33de	19.93cd	45.17	42.50	22.73	1.86c	0.80bc	0.48bc
Pokkali	92.67a	58.23a	29.57a	46.73	39.40	19.30	3.35a	0.98a	0.71a
BR29	48.33cd	24.00e	21.33b-d	45.73	35.43	15.10	1.33d	0.70cd	0.55b
CV (%)	4.54	8.57	11.17	5.5	7.2	4.5	6.9	7.75	8.61

Table 4. Effect of NaCl on proline and sugar content in rice seedlings

Cultivars	Proline			Sugar content		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	4.41±0.09	3.42±0.58	2.76±0.14	43.0±2.11	51.20±2.11	28.35±0.03
MR211	1.03±0.16	1.59±0.04	1.53±0.34	36.21±2.72	43.04±0.33	20.81±0.37
MR219	6.33±0.29	7.17±0.63	1.18±0.01	60.88±0.57	54.07±5.36	21.81±1.05
MR220	4.49±0.07	2.63±0.25	2.82±0.39	76.68±10.51	50.62±5.92	30.99±9.15
MR232	3.75±0.51	4.95±0.045	2.67±0.28	59.74±6.36	71.35±9.84	46.16±2.99
Pokkali	1.53±0.12	1.88±0.01	1.33±0.11	64.89±3.15	55.18±6.68	24.95±1.70
BR29	45.73	35.43	15.10	1.33d	0.70cd	0.55b
CV (%)	5.5	7.2	4.5	6.9	7.75	8.61

and MR211 while it was minimum in BR29.

Proline content: The amount of proline substantially increased in plants under salt stress and generally followed an increasing trend except for MR84 and MR232 (Table 4). There was a significant variation in proline content among all rice cultivars. MR211 and MR185 genotypes accumulated the maximum amount of proline while BR29 and MR220 genotypes had the minimum amount of proline in their shoot tissue. MR 211 cultivar showed high amounts of proline in both salt-stressed and control treatments and is also tolerant to salinity stress (Table 4). These results are similar to Danai-Tambhale et al. (2011) but contradictory to Momayezi et al. (2009). Momayezi et al. (2009) have reported that the highest accumulation of proline was detected at a salt concentration of 5 dS m^{-1} and a decreasing tendency was noted beyond this point. Moreover, there was no proven correlation found between the growth metrics and proline accumulation (Momayezi et al., 2009). The seed priming with varying concentrations of proline significantly enhanced the germination (%), seed vigour index, and α-amylase activity of rice genotypes under both normal and salinity conditions (Singh et al., 2018). The beneficial effect of seed priming with proline on various traits was more pronounced under salinity than in normal conditions.

Sugar content: The salinity stress increased sugar content in MR211 and MR232, while sugar content was decreased in MR84, MR219, MR220, Pokkali, and BR29 (Table 4). The total sugar in the treated seedlings was reduced to 48%. The accumulation of soluble sugars in response to salinity is quite well documented in many plant species (Dubey and Singh 1999, Pattanagul and Thitisaksakul 2008). While the rise in sugar buildup in reaction to salinity stress was only observed in salt-sensitive cultivars (Pattanagul and Thitisaksakul 2008), it was also observed in MR211, a cultivar that is moderately tolerant to saline conditions (Table 4). The under salinity stress accumulation of sugars along with other compatible solutes contributes to an osmotic adjustment allowing the plants to maximize sufficient storage reserves to support basal metabolism under a stressed environment (Dubey and Singh 1999). Soluble sugars may function as a typical osmo protectant, stabilizing cellular membranes and maintaining turgor pressure.

CONCLUSION

Rice has higher salt tolerance during the seed germination phase compared to the initial seedling stage, like other crops like barley, wheat, and triticale. Contemporary rice varieties, except BR29, show higher salt tolerance during the early stages. Evaluating salt resistance during advanced growth phases is crucial for accurate rice cultivars, which is currently underway in our research.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the conception, execution, data collection, data analysis, writing, and critically revised the manuscript, and approved the final version.

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