Impact of Different Types and Levels of Salinity on Early Germination and Growth Parameters of *Oryza sativa* **L. Cv. MR219 seedlings**

***Syeda Maasooma Zahra¹ , Mohd Hafiz Ibrahim1, Rosimah Nulit¹ , Muhammad Nazmin Yaapar ²**

¹Department of Biology, Faculty of Science, University Putra Malaysia 43400 Serdang, Selangor ²Department of Crop Science, Faculty of Agriculture, University Putra Malaysia 43400 Serdang, Selangor

* Corresponding author. Tel.: +60 14-2840743; e*mail address*: maasoomazahra@yahoo.com maasoomazahra@yahoo.com

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ABSTRACT

In order to study salt tolerance in rice (Oryza sativa L.) at early germination stages, MR219 seeds were tested to check tolerance to salinity stress at five different levels which included 50, 100, 150, 200 and 250mM and distilled water was used as control (0mM) with Completely randomized design (CRD) with four replicates. Data were analyzed by two-way Analysis of Variance method (ANOVA), using a Statistical Analysis System (SPSS window, version 22). MR219 seeds were surface sterilized before the experiment. Data were recorded for 14 days focusing on different traits like root and shoot length, fresh weights, biomass, water uptake percentage, germination percentage, seed vigor index, relative injury rate, mean germination time and salt tolerance (ST). Results from the above-mentioned traits showed that salinity affected almost all parameters. MR219 seeds were tolerant to salinity, except 200mM and 250mM of NaCl and 250mM MgSO⁴ because no germination was observed in these 3 levels that could be seen in relative injury rate due to high level of salinity. Thus, it was proposed in the current experiment that salinity order for MR219 was NaCl then KCl followed by MgSO⁴ and on the basis of physiological characteristics, MR219 was sensitive to higher saline levels.

Keywords: Rice varieties, Salinity levels, MR219 and Physiological parameters.

INTRODUCTION

The term salinity can be defined as the existence of high concentration of soluble salts in water and soil (Talei *et al*., 2013; Zahedi *et al*., 2011). According to the Food and Agriculture Organization (FAO) (2006) salinity is a current major issue that includes almost worldwide and it is increasing day by day causing a loss in crop productivity. Salinity is a major factor that limits agricultural yield of approximately 20% of the cultivated as well as irrigated area globally. Salt stress effect physiological and biochemical attributes of plants that causes significant reduction in plant yield. The impact of salinity on plant growth and development can be related to change in

morphology, anatomy and metabolism (Molassiotis *et al*., 2005; Amirjani, 2010, but adjustment of these parameters greatly depends upon the degree of damage, duration of stress exposure and plant species (Amirjani, 2010). Salt stress connected with high NaCl that mostly disturbs the growth and yield by dropping the water holding capacity and damage the normal metabolism (Akhtar *et al*., 2001; Akram *et al*., 2001). NaCl salt easily dissolves in water and plays a vital role in ionic balancing in higher plants as well as in crops like rice (Nishimura *et al*., 2011). Processes such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set are adversely affected by high salt and water stress, ultimately causing diminished economic yield and decreased quality of produce.

Potassium chloride is known as metal halide salt used in agriculture for crops as it contains a large number of inorganic sources of potassium (Asghar, 2017). On the other hand, chloride is an important micro nutrient responsible for optimal growth of plants (Badar-uz Zaman *et al*., 2012). KCl also has been proved as a plant enhancer for growth and mean germination time in saline conditions of rice plants (Afzal *et al.*, 2012). K^+ is essential for many biochemical and physiological procedures that are accountable for plant growth and development. K^+ takes part in protein synthesis, carbohydrate metabolism, and enzyme activation (Wang *et al*., 2013). Potassium assists in seed germination by initiating the rapid imbibition of water, and it also facilitates other physiological processes (Farooq *et al*., 2008).

Magnesium (Mg) has been known forits essential role in chlorophyll development and photosynthesis process in plants. Mg plays a crucial role in the living system due to its essential role because it is one of 18 nutrients that is important for plant growth.

Rice is a type of grain that has shaped the cultures, diets and economies of billions of Asians. Salt stress leads to reduction in limiting agriculture production and causing adverse damage to plant growth (Lauchli and Grattan, 2007). Tolerance to salinity under different substrate with a high amount of soluble salts is the specific ability for plants in their life span (Parida and Das, 2005). Every plant is sensitive to specific salt concentration. The limiting concentrations change with plant species, variety and stage of development and duration of the salt stress (Eynard *et al.*, 2005). Plants subjected to salt stress display complex responses. The response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity (Lutts *et al.*, 1996; Zeng and Shannon, 2001). Salt stress is a main abiotic stress at almost all plant growth stages of rice due to anthropogenic contributions to global warming. In the near future, there might be a chance ofsea level increase in coastal areas that might cause a melodramatic effect on rice production (Hakim *et al*., 2013). MR219 rice variety was used in this study and is a Malaysian Indica rice variety as the product is a cross between MR137 and MR151 from the Malaysian Agriculture Research and Development Institute (MARDI) in 2001.

The researcher carried out their research on various adaptive responses to salinity stress at molecular, cellular, metabolic, and physiological levels, although mechanisms underlying salinity tolerance are far from being completely understood (Hasanuzzaman *et al*., 2013). Therefore, this crisis attracts many scientists to gain their interest in developing salt tolerant rice cultivars to prevent the unnecessary loss in agriculture and at the same time reduce food shortage problems (Hakim *et al*., 2014). Therefore, the purpose of this study was to identify the impact of salinity on early seedling growth of MR219 with different salts and various salinity levels.

MATERIALS AND METHODS Plant Material and Seed Sterilization

The experiment was conducted in the laboratory of the biology department, faculty of science, University Putra Malaysia during the period of July 2018 to September 2018. Seeds of *Oryza sativa* cv. MR219 rice variety was used in the study and collected from the Ministry of Agriculture Malaysia. Healthy, uniform seeds of *Oryza sativa* cv. MR219 sterilization was done according to a report by Nwe, Maziah, Ho, Faridah, and zain (2011) with slight modifications. Healthy seeds were selected and surface sterilized with 70% ethanol solution for 30 sec. Seeds were washed in 5% sodium hyperchlorite (NaoCl) solution containing one drop of Tween 20 for another 20 minutes. After that seeds were washed thoroughly with autoclave distilled water and followed by air-dried with tissue paper.

Experimental design and Salt Treatments

Types of salts used were KCl (Univar, Austrailia), MgSO⁴ (Bio Basic, Canada) and NaCl (Sigma USA) was used to prepare the salt solution because it was safe without causing any precipitation of different ions. 10 sterilized seeds were spread and allowed to grow on NICE filter paper in 9 cm-diam sterilized Petri dishes. Filter paper was moistened with salt solution of 0 (deionized water) as a control followed by 50, 100, 150, 200, and 250 mM salt concentrations. 7 ml of appropriate solution was applied to each Petri dish. The Petri dishes were arranged in completely randomized design (CRD) with four replications for each treatment. Germination room temperature was maintained 25 ± 1 °C with 12 h daylight. The number of seeds that sprouted and germinated was counted daily up to 14 days. All petri dishes were covered with parafilm to avoid contamination.

Observation and Data collection

Seedling shoot and root length of 5 randomly selected seedlings from each replication were measured at the time of harvest with a scale. Shoot dry weight and root dry weight were recorded after oven drying at 70°C for 72 h. After final count, water uptake percentage (Gairola *et al*., 2011), germination percentage (Kandil *et al*., 2012), germination index and relative injury rate (Li, 2008), mean germination time (Ellis & Roberts, 1981), Biomass (Carpýcý *et al*., 2009), Salt tolerance Tsegay & Gebreslassie, 2014) and Seedling height reduction (Islam & Karim, 2010) were measured

Statistical Analysis

The data obtained were subjected SPSS window version 22 to a two-way analysis of variance to determine the differences between treatments and varieties, and when significance occurred, means were compared using Duncan's multiple range test at p<0.05 significance level.

RESULTS AND DISCUSSIONS

Root Length (RL) and Shoot Length (SL). The ANOVA results showed that salinity leads to reduction in root length in seedlings of MR219 (p < 0.05). It is observed that an increasing salt concentration levels lead to significant reduction in root length as shown in Table1. Results showed that after control MR219 showed better seedling germination in 50mM and 100mM solution of salt for NaCl and KCl as compared to rest levels. As for NaCl and MgSo₄ no germination was observed in 250mM due to high salinity. On the other hand, KCl showed slight germination at this

level. However, overall MgSO⁴ was responsible for the least germination for MR219 at all levels. Overall, root growth reduced drastically with treatment application. Negative impact of salinity can be seen more on shoot length as compared to root growth (Table1). Statistical results showed that reduction in shoot length were observed with increasing salinity level $(p<0.05)$, however, all levels were significantly different from each other. Seedlings of MR219 did not show any growth in 200mM and 250mM due to high salinity levels. While shoot length of rice seedlings under MgSO⁴ suppressed in all levels as compared to KCl and NaCl. Surprisingly, rice seedlings under KCl treatment showed shoot length growth even in 250mM.

Table 1

Impact of different types and levels of salinity on root length and shoot length of Oryza sativa L*. cv. MR219 seedlings*

Note. Values are mean \pm standard error (n=4) means with a column that have different superscript letters (a-l) are significantly different from each other (Duncan multiple rang test, $p < 0.05$).

Plant Fresh weight (PFW) and Plant Dry Weight (PDW). It was observed that seedlings fresh and dry weight reduced as salt concentration increased (Table 2). For PFW after 50mM showed higher growth after control, while no significant difference was observed in NaCl 150mM and 100mM of MgSO₄ for PFW. Statistical analysis ($p<0.05$) for Table 2 showed that increasing salt concentration leads to reduction in PDW. NaCl 50, 100 and 150mM showed more PDW as compared to control. No significant (ANOVA $p<0.05$) difference was found for 50, 100 and

150mM MgSO4. Findings show that KCl 50mM was the ideal concentration for MR219 seedling fresh weight. Overall, dry weight reduction for plant can be done due to slow mobilization of food reserves that suspend cell division, enlarging and injuring hypocotyls with an increase in salt concentrations.

Table 2

Impact of different types and levels of salinity on plant fresh and dry weight of Oryza sativa L. *cv. MR219 seedlings*

Salts	Levels		Parameters
(mM)		PFW	PDW
NaCl	control	0.062 ± 0.000 a	0.015 ± 0.000 c
	50	0.043 ± 0.001 c	0.017 ± 0.000 b c
	100	0.033 ± 0.001 e	0.017 ± 0.001 b
	150	0.028 ± 0.000 fg	0.020 ± 0.000 a
	200	0.012 ± 0.000 k	0.011 ± 0.000 d
	250	$0.012 \pm 0.000 \text{ k}$	0.011 ± 0.000 d
KCl	control	0.062 ± 0.000 a	0.015 ± 0.000 c
	50	0.048 ± 0.001 b	0.016 ± 0.001 b
	100	0.038 ± 0.000 d	0.012 ± 0.000 d
	150	0.030 ± 0.001 f	0.011 ± 0.000 d
	200	0.025 ± 0.000 h	0.011 ± 0.000 d
	250	0.150 ± 0.001 j	0.011 ± 0.000 d
MgSO ₄	control	0.062 ± 0.000 a	0.015 ± 0.000 c
	50	0.035 ± 0.001 e	0.017 ± 0.000 bc
	100	0.027 ± 0.001 gh	0.016 ± 0.000 bc
	150	0.025 ± 0.001 h	0.016 ± 0.001 bc
	200	0.020 ± 0.000 i	0.012 ± 0.000 d
	250	$0.018 \pm 0.000 i$	0.011 ± 0.000 d

Note. Values are mean \pm standard error (n=4) means with a column that have different superscript letters (a-i) are significantly different from each other (Duncan multiple rang test, $p<0.05$)

Mean germination time (MGT) and Water uptake percentage (WU%). Higher salt concentration leads to the extended germination period until seeds grow fully tolerant and start to germinate by adapting to overcome the germination time interruption. Table 3 shows that mean germination time (MGT) for MR219 seedlings increased as the salinity level increased until three levels 50, 100 and 150mM except 200 for NaCl. This shows that salinity has a significant growing effect on germination time of MR219 seed up to a specific level. That means, the higher the salinity level, more germination time is needed until seeds develop tolerance and start to germinate. On average, the maximum mean germination time for NaCl (150mM) was much higher as compared to other concentration. Meanwhile, no germination was observed for 250mM MgSO⁴ and NaCl, this is because of the increasing osmotic potential to decrease water uptake and leads to slowing down germination time (Uhvits, 1946; Simon, 1984; Werner and Finkelstein, 1995). On the other hand, water uptake (WUP) is an essential condition for the commencement and completion of seed

germination. H_2O , O_2 and a suitable temperature are essential and adequate for a developed, nondormant seed to fulfil its germination (https://extension.psu.edu/seed-and-seedling-biology). Statistical analysis showed significant differences among salinity types (ANOVA, p<0.05). Water potential uptake was greater under KCl salinity levels and reduced under NaCl salinity levels. A gradual decrease in water potential uptake was noticed as salinity levels increased from 50mM to 250mM respectively. The salinity level of 150mM of each salt was found most effective for water potential uptake in *Oryza sativa* L. Cv. MR219. Water uptake percentage decreased with increasing salinity level.

Table 3

Impact of different types and levels of salinity on Mean Germination rate (MGT) and Water Uptake Potential (WUP) of Oryza sativa L*. cv. MR219 seedlings*

Note. Values are mean \pm standard error (n=4) means with a column that have different superscript letters (a-k) are significantly different from each other (Duncan multiple rang test, $p<0.05$)

Germination Percentage (GP) and Seed Vigor Index (SVI). High absorption of sodium and chloride ions during seed germination can cause cell toxicity and eventually inhibits the rate of

germination that leads to the decrease in germination percentage. Growth percentage statistical data (ANOVA, p<0.05) of *Oryza sativa* L. Cv. MR219 revealed non-significant differences for NaCl and KCl salinity levels. There was no difference in growth percentages among different salinity under control conditions. The highest growth percentage was measured for KCl at all salinity levels from 50mM to 250mM respectively. Among NaCl and MgSO₄ salinity levels, no growth percentage was recorded at 250mM while the lowest growth percentage was noticed for 200mM NaCl salinity level. Salinity level of 150mM of all salinity types showed the best growth percentage as compared to 50mM and 100mM respectively. Seed vigor is characterized as a potential determinant for rapid and uniform seedling emergence and crop establishment. Results for seed vigor index were found highly effective for all types of salinity as well as salinity levels. A gradual decrease in seed vigor index was noticed as salinity levels increased from 50mM to 250mM respectively (Table 4). The highest seed vigor index was measured for NaCl at all salinity levels from 50mM to 250mM respectively. Among NaCl and MgSO⁴ salinity levels, no seed vigor index was recorded at 250mM while the lowest growth percentage was noticed for 250mM KCl salinity level. Salinity level of 100mM of all salinity types showed best seed vigor index as compared to 50mM respectively. Seed vigor decreased with increasing salt level.

Table 4

Impact of different types and levels of salinity on Germination Percentage (GP) and Seed Vigor Index (SVI) of Oryza sativa L*. cv. MR219 seedlings*

Note. Values are mean \pm standard error (n=4) means with a column that have different superscript letters (a-l) are significantly different from each other (Duncan multiple rang test, $p<0.05$)

Relative injury rate (RIR) and Salt tolerance (ST). RIR was used to determine the degree of injury on any plant during germination stage was caused by salinity. Relative injury rate data showed significant differences for all salinity types and levels. There was a direct relationship between salinity levels and injury rate as when the level increased, the injury also increased. Findings showed that the lowest relative injury rate was measured in control as compared to all salinity types. An increase in relative injury rate was recorded with an increase in salinity level from 50mM to 250mM for NaCl and MgSO⁴ respectively. KCl showed the least relative injury rate at all salinity levels as compared to NaCl and MgSO₄ (Table 4). Previous study showed an increment in RIR of MR219 rice seedlings (Tsegay and Gebreslassie, 2014).

Rice are tolerant during germination but becomes susceptible to salinity during early seedling development (Singh *et al*., 2009). Statistical results for salt tolerance revealed significant differences for all salinity types and levels. The lowest salt tolerance was measured in control as compared to all salinity types. The maximum salt tolerance was recorded for NaCl and MgSO⁴ with an increase in salinity level from 50mM to 250mM respectively (ANOVA, p<0.05). KCl showed the least salt tolerance at all salinity levels as compared to NaCl and MgSO4. Salt tolerance screening at germination offers little basis for further crop-salt tolerance because most germination studies are conducted in a laboratory where it is easy to observe the seed germination process as compared to the field, so salt tolerance may differ in every environment (Lauchi and Grattan, 2007). Salinity normally delays despite the tolerance during germination.

Table 5

Salts	Levels	Parameters		
	(mM)			
		RIR	ST	
NaCl	Control	0.000 ± 0.000 j	$0.000 \pm 0.000 e$	
	50	0.075 ± 0.025 hi	113.2 ± 1.202 bc	
	100	0.200 ± 0.000 def	$113.8 \pm 3.782 b$	
	150	0.075 ± 0.025 hi	131.2 ± 1.351 a	
	200	0.750 ± 0.086 b	74.52 ± 0.000 d	
	250	1.000 ± 0.000 a	74.52 ± 0.000 d	
KCl	Control	0.000 ± 0.000 j	$0.000 \pm 0.000 e$	
	50	0.100 ± 0.001 gh	77.41 ± 2.082 d	
	100	0.150 ± 0.028 fgh	78.18 ± 0.706 d	
	150	0.175 ± 0.025 efg	71.81 ± 4.544 d	
	200	0.250 ± 0.028 cd	71.65 ± 1.592 d	
	250	0.200 ± 0.000 def	72.13 ± 0.656 d	
MgSO ₄	Control	0.000 ± 0.000 j	$0.000 \pm 0.000 e$	
	50	0.125 ± 0.025 fgh	109.3 ± 0.000 bc	

Impact of different types and levels of salinity on Relative injury rate (RIR) and Salt tolerance (ST) of Oryza sativa L. *cv. MR219 seedlings*

Note. Values are mean \pm standard error (n=4) means with a column that have different superscript letters (a-j) are significantly different from each other (Duncan multiple rang test, $p < 0.05$)

Apart of growth conditions, effect of salinity on leaf color of *Oryza sativa* L. cv. MR219 seedlings can be seen in table 6**.**

Table 6

Impact of different types and levels of salinity on growth conditions and leaf color of Oryza sativa L*. cv. MR219 seedlings*

Treatment	Growth Conditions	Colour of leaf
control	Normal	Green
	NaCl	
50 _m M	Normal	Green
100mM	Normal	Green
150mM	Moderate	Yellowish green
200mM	Less	Yellowish green
250 _m M	Very less	Yellow
	KCl	
50 _m M	Normal	Yellowish green
100mM	Normal	Slight yellowish
150mM	Moderate	Slight yellow
200mM	Suppressed root growth	Yellowish green
	Unusual, suppressed root	
250mM	growth	Yellowish green
	MgSO ₄	
	Unusual, suppressed root	
50 _m M	growth	Yellowish green
	Unusual, suppressed root	
100mM	growth	Yellowish
	Unusual, suppressed root	
150mM	growth	Brownish yellow
	Unusual, suppressed root	
200mM	growth	Brownish yellow
	Unusual, suppressed root	
250 _m M	growth	Brownish yellow

DISCUSSION

Due to sudden changes in saline environment, NaCl has been widely used in many researches to study seed germination. This opened up interest by other researchers to study the application of other salt types on the germination stage and early growth on different plants. In the present study, three salts namely NaCl, KCl and MgSO⁴ effect werecompared on *Oryza sativa* L. cv. MR219 seedlings, the results showed rice seedlings responded significantly different to various salinity levels (Table 6). Two-way analysis of variance (ANOVA) related to root and shoot length showed significant results. However, SL and RL of MR219 seedlings were badly effected with increasing salinity level that led to the reduction in root and shoot length. Reduction of shoot length is a common phenomenon of many crop plants under salinity stress as observed earlier by several researchers (Javed & Khan, 1975; Amin *et al*., 1996).

Under salt stress, the plant suffersfrom osmotic stress. For adjustment purpose, the plant has to osmotically produce suited metabolites that eventually need additional energy. This is the reason why plant growth suffers significantly (Taiz and Zaiger, 1991). Karim *et al*. (1992) pointed out that shoot growth was more sensitive to salinity than germination and root growth. Current results showed that root growth was suppressed by salinity and shoot length of all salts at high salinity levels were considerably affected especially in MgSO4, where all levels showed suppressed root growth except 50mM. Mg^{2+} is an essential micro nutrient for plants. Furthermore, magnesium involved in photosynthesis helps in making central atom in chlorophyll and acts as a metallic cofactor for enzymes (Brandenburg and Kheier, 2011). Surprisingly in all levels, KCl maintained to produce root growth even under 250mM. It had delayed germination period but was able to produce as compared to NaCl and MgSO₄. K^+ serves as an agent that helps in osmotic adjustments and helps in protein and starch synthesis by acting as a metabolic agent (Zehra *et al*., 2013). For NaCl and MgSO4, only 50mM and 100mM were able to produce effectively while 150mM and 200mM had very less growth for both shoot and root length. (Table 1).

The findings of the current experiment are similar with the findings of Cramer *et al*. (1985). Salt stress roots play a key role by excluding $Na⁺$ salt or by directing an easy pass of $Na⁺$ to the shoot (Karim *et al.*, 1992; Amin *et al.*, 1996). However, a seed does not need a large amount of Mg^{2+} in germination stage as it can lead to some abnormalities in rice seedlings even at lower salt application. NaCl and KCl suggest that Na and K are less harmful as compared to Mg and the same finding was revealed by Tobe *et al.* (2002). Lower root growth in MgSO₄ due to SO⁴²⁻ ion is believed to enhance the suppressive effect of root growth and combined application of Mg^{2} and So₄² in the form of MgSO₄ could lead to more damage to roots as compared to shoot. More root damage means less chances for plant water conductance because roots are the tools for water and mineral absorptions.

Ion toxicity caused root injury that led to death and in result, WUP affected in plants that increased deficiency of water in plants causing a reduction in photosynthesis and this led to the reduction in shoot growth. Water shortage may happen due to the reduction in water potential of the soil solution and limiting root water uptake. Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation (Netondo *et al.,* 2004). Shoot length, root lengths and dry weights of paddy were decreased with

increasing salt stress. The reduction in root and shoot development may be due to toxic effects of NaCl as well as unbalanced nutrient uptake by the seedlings (Datta *et al.,* 2009).

CONCLUSION

It was concluded that germination and early seedling growth of different rice varieties were inhibited by increasing salt concentration. MR219 seeds had different responses under different salinity levels during germination and early seedlings stage. Increased salt concentration led to the reduction in seed vigor index, germination percentage, relative injury rate, mean germination time and water uptake percentage of rice seedlings. KCl showed better performance in all salinity levels, even in 200mM and 250mM as compared to MgSo4. Based on the overall results, we concluded that MR219 is sensitive to high salinity level. In terms of salinity tolerance, we observed that the order for MR219 is NaCl<KCl<MgSO₄.

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