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Effect of NaCl Induced Stress on Germination and Seedling Growth of Various *Oryza sativa* L. Genotypes

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Authors' contributions

This research work was performed by all authors. Author AMG, gave the research design and wrote the first manuscript draft. Author AA, carried out the research work and manuscript writing. Author MA, performed the statistical analysis and manuscript writing. Author AE, contributed in some literature and manuscript writing. All authors read and approved the finial manuscript.

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ABSTRACT

An experimental study aimed to investigate the effect of saline stress on rice (*Oryza saliva* L.) germination and early seedling characteristics, and genotypic differences in response to saline stress was conducted under lab conditions at College of Food and Agricultural Sciences, King Saud University, Saudi Arabia. Germination percentage (%), germination rate, emergence energy (%), germination speed, seedling height (cm), vigour index, seedling fresh and dry weights (mg) were recorded. Treatments consist of three different saline stress levels: 0 mM (Control), 100 mM

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and 200 mM, and eight rice genotypes; Basmati 385 and Super Basmati (Pakistani), Sakha 101, Sakha 102, Sakha 103, Sakha 104, Sakha 105 and Sakha 106 (Egyptian). It was conceived from results that saline stress significantly affected all the germination parameters in reverse order. (0 mM < 100 mM < 200 mM). Genotypic differences among rice cultivars germinating under saline stress were also recorded significant. Most valuable outcome of the study: interaction between various levels of saline stress and rice genotypes were highly significant. Sakha 101, Sakha 103, Sakha 106 and Basmati 385 have performed better even under 200 mM NaCl; they have higher level of saline stress tolerance potential and could be used in future breeding programs.

Keywords: Germination; rice genotypes; NaCl stress.

1. INTRODUCTION

Abiotic stresses; drought, high and low temperatures, heavy metals and salinity are exercising drastic effects on crop biochemical processes, plant growth and development which result in low agricultural productivity and food insecurity. Among all stresses, salinity is the major crop production limiting factor globally; it affects land cultivation, plant physiological processes and yield [1,2]. Despite a number of advances in biotechnology and crop improvement techniques for pests and diseases, salt stress remained elusive due to the fact that it is a complicated biochemical and physiological phenomenon. Rice after wheat is considered as the 2nd major cereal crop for more than 50% of the world's population. Almost 25% of the recent rice production, 1% annual increase, is required to meet the growing demands of worryingly increasing population [3]. Salt stress adversely affected various plant processes e.g. seed germination, seedling growth, vegetative growth and vigor, flowering percentage, fruit setting and ripening which ultimately resulted in reduced economical production and food quality. Generally, salt stress affects plant growth and germination through ionic imbalance, oxidative compounds, ion-specific effect, osmotic stress and reactive oxygen species [4].

A significant decline in total germination, germination speed leading to reduction in shoot and root length as well as dry biomass accumulation by saline stress has been reported [5]. Seed germination is one of the important factors in crop production; poor and uneven germination derived an inhomogeneous seedling growth and ultimately the economic losses [6]. Despite its sensitivity to salinity, valuable variations have been observed in rice for salinity tolerance. Numerous investigations have reported differences among rice cultivars under saline stress [7-10]. Interactions of NaCl (Salinity) concentrations and rice genotypes at early stage for saline tolerant screening and breeding programs are inevitable. However, salt stress responses may depend upon soil physio-chemical properties, salt concentrations, environmental conditions and genetic information. Therefore, a screening study for salt tolerant genotypes under controlled and uniform conditions is an effective tool to reveal the mysteries of salt tolerance mechanisms, salt toxicity limits and varietal selection [1]. The present investigation focused on comparative effects of NaCl concentrations (Salt Stress) on germination characteristics and early seedling response of *Oryza sativa* genotypes.

2. MATERIALS AND METHODS

Eight rice genotypes; two from Pakistan (Basmati 385 and Super Basmati) and six from Egypt (Sakha 101, Sakha 102, Sakha 103, Sakha 104, Sakha 105 and Sakha 106) were used in germination tests to evaluate their potential saline stress tolerability under three different Sodium Chloride (NaCl) concentrations: 0.0 mM (Control), 100 mM and 200 mM. Initial seed moisture contents were maintained from 8 to 9% on dry weight bases. Healthy and uniform seeds were selected on physical appearance and germination percentage was tested as a prerequisite. Computed seeds for all genotypes were surface sterilized by 1.0% Sodium Hypochlorite aqueous solution for 5 minutes to prevent the fungal growth and then washed rapidly four times with deionized water. Thirty seeds of each genotype were allowed to germinate on filter paper (Whatman # 41) in a 9 cm diameter Petri dish for all three NaCl concentration, including control (0.0 mM). Filter papers were kept moist with equal volume of NaCl solutions for all petri dishes during experiment. Experiment was arranged in Randomized Complete Block Design (Factorial) according to [11] with three replications, were placed at room temperature (25±2°C) and 12 hours of light. Petri dishes were examined on daily bases (after every 24 hours) for twelve days after sowing and seeds were considered germinated when the radical was at least 2 mm in length. Germination characters were recorded 12 days after the starting date. Total germination percentage (%) was computed according to [12]:

$$\text{Germination}(\%) = \frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds Tested}} \text{ X } 100$$

Germination rate (GR) was calculated according to the equation described by [13].

$$\text{Germination Rate (GR)} = \frac{\text{Number of Germinated Seeds}}{\text{Number of Germination Days}}$$

Emergence Energy (EE) is the percentage (%) of germinated seeds 3 days after germination started and total number of seeds tested per treatment. It was computed 72 hours after the start of germination by using following expression of [14]:

$$\text{Emergence Energy (EE)} = \frac{\text{No. of Seedlings Emerged After 72 Hours}}{\text{Total Number of Seeds Sown}} \text{X100}$$

Germination speed (GS) is the percentage (%) of seeds germinated after 3 days of sowing to the 6 days of sowing. It was calculated according to the equation derived by [15]:

$$\text{Germination Speed (GS)}(\%) = \frac{\text{No. of Seeds Germinated at 72 Hours}}{\text{No. of Seeds Germinated at 144 Hours}}$$

Seedling length (cm) was measured 15 days after the sowing with a simple laboratory scale. Whereas seedling vigor was computed by following formula by [16]:

$$\text{Seedling Vigor} = \text{Germination (\%) X Seedling Length (mm)}/100$$

Seedling fresh weight (g) was measured 15 days after sowing; fresh seedlings were harvested and weighed immediately by a digital electronic balance. To determine the seedling dry weight (g), seedlings were dried at 70°C until the constant weight was observed. The data were statistically analyzed by using computer-based program: SAS 9.1. Differences among treatment means at 5% probability level were separated according to [17].

3. RESULTS AND DISCUSSION

Saline stress (NaCl concentration) has adversely affect the germination and vigors parameters in the rice genotype showed that as the stress level increases from control to 200 mM several changes are occurs in all the observations individual as well as interaction (S x G). Highest mean values were found in control (0.0 mM NaCl) while lowest mean values were recorded under control condition. NaCl has direct inhibitory effect on the rice genotypes as the concentration increased resistance against salinity decreases. Hampered rice seedling parameters in relation to NaCl concentration were in order of 200 mM>100 mM> 0.0 mM. Maximum germination percentage (83.12) was found in control while minimum was observed (21.60) at maximum salinity stress (200 mM NaCl). As the NaCl concentration increasing, emergence energy reduced significantly (Table 1). Seedling length showed inverse relationship with increased salt concentration. A decreasing trend with increasing NaCl concentration was observed in vigor index and a remarkable change was observed in seedling weight (Table 1). Decreased in the seedling length is a common phenomenon in many crops including rice with increasing salt level [18]. Seedling length was more suppressed and gradually decreased in root length with increase in salinity.

Germination is a complex physiological phenomenon affected by a number of associated processes i.e. water uptake, osmotic potentials, cell membrane regulation, metabolic activities and genetic information of the seed. Increase in NaCl concentration the germination percentage, energy emergence rate and germination speed of rice varieties were observed (Table 1) similar results were reported by [8,19]. Due to salinity stress metabolic activities was disturbed by which germination speed was reduced by osmotic stress which is common at high salt levels. Seedling height, vigor index and seedling weights (fresh & dry) are associated with plant health, water availability, root osmotic potential and metabolic activities [20].

Table 1. Effect of saline stress and genotypic difference of germination and early seedling characteristics of rice

Treatments		Germination (%)	Germination rate	Emergence energy (%)	Germination speed (%)	Seedling length (cm)	Vigor index	Seedling fresh weight (mg)	Seedling dry weight (mg)
NaCl conc.	0.0 mM	83.12 A	4.1169 A	70.82 A	75.17 A	10.15 A	8.56 A	67.62 A	10.50 A
	100 mM	63.07 B	2.5543 B	29.52 B	40.29 B	03.24 B	2.18 B	54.51 B	8.79 B
	200 mM	21.60 C	0.7691 C	5.32 C	31.38 C	0.71 C	0.16 C	46.44 C	7.63 C
	LSD	1.0375	0.0375	0.7537	1.8016	0.1707	0.630	0.9167	0.8968
Rice genotype	Sakha 103	61.90 B	3.1097 A	42.00 C	54.02 C	4.64 C	3.71 CD	50.62 D	10.91 A
	Super Basmati	30.41 F	1.7282 H	19.77 G	40.14 E	2.70 E	1.57 F	39.95 E	6.19 D
	Sakha 101	66.30 B	2.8139 C	44.94 B	57.84 B	4.90 C	3.82 C	64.50 A	9.17 BC
	Sakha 102	54.63 D	2.4416 F	31.01 E	39.04 EF	3.20 D	2.26 E	56.43 C	9.28 B
	Sakha 106	66.19 A	2.9778 B	48.24 A	64.80 A	6.06 A	5.07 A	56.99 C	9.76 AB
	Sakha 104	63.52 B	2.5917 D	39.31 D	49.02 D	5.57 B	4.53 B	65.94 A	9.57 AB
	Basmati 385	49.85 E	1.8178 G	27.76 F	50.18 D	4.86 C	3.61 D	51.86 D	7.92 C
	Sakha 105	58.62 C	2.4416 E	28.70 F	36.54 F	5.67 B	4.52 B	62.27 B	9.14 BC
	LSD	1.6943	0.0613	1.2308	2.9420	0.2788	0.624	1.4970	1.4644

Means not sharing common letters at 5 % probability level

Table 2. Effect of saline stress and genotype interaction on germination and early seedling characteristics of rice

Treatments		Germi-nation (%)	Germination rate	Emergence energy (%)	Germination speed (%)	Seedling length (cm)	Vigor index	Seedling fresh weight (mg)	Seedling dry weight (mg)
NaCl conc.	**Rice genotypes**								
0.00 mM	Sakha 103	86.003 B	5.289 A	82.593 A	85.157 A	09.780 E	8.44 E	60.801 EF	13.32 A
	Super Basmati	67.883 F	2.456 I	53.330 G	67.116 DE	06.623 F	4.49 G	58.778 F	8.48 DEFG
	Sakha 101	83.330 C	4.103 D	73.220 C	88.547 A	11.507 C	9.58 C	75.255 A0	10.41 BCDE
	Sakha 102	75.670 E	3.853 E	63.556 F	70.183 CD	7.053 F	5.33 F	66.037 C	10.36 BCDE
	Sakha 106	88.003 B	5.238 A	81.889 A	88.265 A	13.760 A	12.11 A	75.050 A	12.29 AB
	Sakha 104	86.447 B	4.317 C	75.776 B	76.513 B	12.007 B	10.38 B	65.570 C	10.31 BCDE
	Basmati 385	85.777 BC	3.180 F	69.722 D	73.553 BC	10.326 D	8.85 D	63.477 CD	8.17 DEFG
	Sakha 105	91.527 A	4.499 B	66.443 E	52.040 F	10.147 DE	9.29 C	73.130 A	10.70 BCD
100mM	Sakha 103	76.290 E	3.250 F	40.485 I	28.026 IJ	3.282 J	2.50 J	45.713 H	11.41 ABC
	Super Basmati	16.443 K	2.466 I	2.931 O	6.732 L	1.147 L	0.19 M	38.140 J	6.96 FG
	Sakha 101	69.780 F	3.053 G	50.999 H	51.930 F	2.253 K	1.58 KL	45.543 H	7.60 FG
	Sakha 102	67.777 F	2.499 I	26.374 K	21.817 K	1.937 K	3.14 L	59.516 F	8.91 CDEFG
	Sakha 106	80.333 D	2.690 H	52.221 GH	63.669 E	3.506 IJ	2.82 IJ	54.337 G	8.71 DEFG
	Sakha 104	73.780 E	2.307 J	36.219 J	45.027 GH	4.077 H	3.01 I	69.438 B	9.23 CDEFG
	Basmati 385	50.117 E	1.843 K	10.485 M	25.129 JK	3.826 HI	1.92 K	54.310 G	8.38 DEFG
	Sakha 105	70.000 F	2.327 J	16.442 L	8.730 L	5.893 G	4.13 H	69.077 B	9.12 CDEFG
200mM	Sakha 103	23.110 J	0.790 O	2.931 O	48.837 FG	0.852 LMN	0.20 M	45.353 H	7.99 EFG
	Super Basmati	06.893 L	0.263 Q	3.056 O	46.573 GH	0.341 O	0.03 M	22.919 K	3.13 H
	Sakha 101	33.777 H	1.285 L	10.610 M	33.037 I	0.927 LM	0.32 M	72.687 A	9.49 CDEF
	Sakha 102	20.443 J	0.729 O	3.111 O	25.111 JK	0.622 MNO	0.13 M	43.747 HI	8.56 DEFG
	Sakha 106	30.220 I	1.005 N	10.597 M	42.477 H	0.920 LM	0.28 M	41.570 I	8.29 DEFG
	Sakha 104	30.333 I	1.152 M	5.943 N	25.533 JK	0.636MNO	0.19 M	62.813 DE	9.16 CDEFG
	Basmati 385	13.893 K	0.430 P	3.073 O	51.893 F	0.420 NO	0.06 M	37.797 J	6.83 G
	Sakha 105	14.333 K	0.499 P	3.216 O	48.837 GH	0.973 LM	0.14 M	44.617 H	7.59 FG
LSD		2.9346	0.1061	2.1318	5.0958	0.4829	0.3573	2.5929	2.5365

Means not sharing common letters at 5 % probability level

Reduction in fresh and dry weight of seedlings was due to poor CO_2 fixation efficiency under saline conditions [21]. It might be due to the toxic effect of NaCl ions, osmotic imbalance or disturbed metabolic activity. Absorption of water and ions is lowered under-saline conditions by which loss of cell turgidity occur [22]. Shoot length, fresh and dry weight are decreased with increased the salinity level. These results are in line [23] reported that with increase in the salinity level the growth of the rice genotypes were suppressed.

Rice genotypes showed great variation under normal to high salt stress level. This variation depicts their tolerance to saline conditions (Table 1). Germination is a physiological process starts with the imbibition of water followed by emergence of radical. Germination is the most critical stage in seedling establishment and determines the successful crop production [24]. Maximum germination percentage was (66.19) observed with Sakha 106 while least was (30.41) observed in super basmati. High germination rate means more seedling establishment and more crop production. Sakha 106 gave better emergence energy (48.24%), speed of germination (64.80) and seedling length (Table 1). Vigor index was used to measure percentage of viability, this is an important to judge the ability of seeds to produce normal plants under different adverse conditions like salt stress. Vigor index have an inverse relation with salinity and highest mean value was recorded for Sakha 106 (5.07) while minimum was observed (1.56) for super basmati Table 1.

Highest seedling fresh weight was resulted for Sakha 104 and Sakha 101, 65.99 mg and 64.50 mg, respectively. Maximum seedling dry weight was recorded for Sakha 103 (10.91 mg) and minimum was recorded for super basmati (Table 2) above. Rice has been considered more sensitive crop regarding salinity. However, rice has huge genetic differences as it has been domesticated centuries ago in different geographic regions by a number of civilizations as a staple food. In last century, many well recognized institutes and research centers have been developed for rice research and their contribution to the varietal development for acquired characters have diversified the rice genome. For all germination and vigor parameters studied in this research, highly significant variations among rice genotypes under saline stress conditions were revealed [25].

Maximum germination percentage was (91.53) observed with Sakha 105 while seedling length and vigor index showed by Sakha 106 at 0.00mM NaCl. Sakha 101 produced more fresh weight followed by Sakha 103 at 200 mM NaCl salinity level (Table 2) above. Sakha 103 produced more dry weight at controlled while least was produced by super basmati (Table. 2) above. Sakha 101 is more resistant under high salinity level while others rice varieties show susceptible behavior under same saline stress regarding germination percentage, germination rate and emergence energy, seedling length, seedling fresh weight and seedling dry weight (Table 1) while basmati 385 have more emergent speed under high (200 mM) saline stress. Sakha 106, Sakha 103 and Sakha 101 are susceptible at moderate 100mM NaCl salinity stress while at maximum level their stability is more diverse although, the differences are lower within the group (Table 1) [18,19,26,27] had reported the genotypic differences in rice for saline stress resistance and suggested to use them for further breeding programs.

4. CONCLUSION

The results of this experiment indicated that saline stress suppressed rice seed germination parameters and early seedling growth and vigor; however, genetic differences were present for salt stress tolerance. Maximum rice seedling parameters were produced by Sakha 101, Sakha 106, Sakha 103 and Basmati 385 up to salinity level of 200 mM NaCl. These cultivars were more tolerant to salinity than others, and therefore must be used in breeding programs to enhance rice commercial verities saline stress resistance and to increase grain production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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