Indian Journal of Plant Sciences ISSN: 2319–3824
Online, International Journal, Available at http://www.cibtech.org/jps.htm
2025 Vol.14, pp.17-22/Shukdeb and Subhas
Research Article (Open Access)

EVALUATING SEED GERMINATION ABILITY OF BREEDING LINES (YAMUNA X *ORYZA RUFIPOGON*) UNDER SALT STRESS

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ABSTRACT
The ability of rice (Oryza sativa L.) to withstand salt during the seed germination phase is one of the key factors that determines the establishment of a stable stand in salinity soil. The germination traits, such as imbibition rate, germination percentage for 5 and 10 days, and root and shoot length under control (water) and different salt stress (50 mM, 100 mM, 150 mM, 200 mM, and 250 mM NaCl) conditions, were measured using tweenty breeding lines of (F_3), which were produced from an interspecific cross between a wild rice *Oryza rufipogon* and a *sativa* rice variety Yamuna. The breeding lines YR106 and YR43 showed highly salt sensitive in seed germination and on the other hand YR11, YR13, YR38, YR76 and YR 88 breeding lines germinate in high salt stress (200mM). Among them YR13 showed higher resistance and germinate 250mM salt concentration.

Keywords: *Rice, Germination, Imbibitions, Salt tolerance*

INTRODUCTION
Soil salinity is a major abiotic stress in crop productivity worldwide (Zhu 2001). Rice is subject to salt stress at various developmental stages, including germination, vegetative, and reproductive growth stages. Improving rice salt tolerance at the germination stage has recently become more significant, as the direct seeding method has grown in popularity in many Asian countries due to its lower cost and ease of operation (Fujino *et al.*, 2004). In rice, the ability of seeds to germinate at various stages is linked to a number of quantitative characteristics, including seed imbibition rate, germination rate, germination speed, shoot length, root length, and seed vigor. A prior study found a negative correlation between imbibition rate and rice seed germination under salt stress; lowering the imbibition rate may enhance seed germination (Wang *et al.*, 2010). It's possible that improved repair mechanisms during seed imbibition are the cause of improved seed germination performance by lowering the imbibition rate under salt stress.

MATERIALS AND METHODS
Evaluation of seed germination under salt stress
A total of fifteen high-quality seeds were collected for each salt concentration and control, and their dry weight was determined. Following a 15-minute surface sterilization with a 0.1% sodium hypochlorite solution, the seeds in each line were washed three times with sterile distilled water. The seeds were then put in petri plates (d = 9 cm) with two sheets of filter paper. 10 mL of distilled water and concentrations of 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM NaCl solution were added, and the seeds were left for a full day. To measure the change in grain weight following water absorption, the seeds were dried on tissue paper and measured. The seeds were again soaked in distill water and 50mM, 100mM, 150mM, 200mM and 250 mM concentrations of NaCl solution for another 24 hours. Using tissue paper, the grain was dried once again, and a pan balance was used to weigh it from distill water and various NaCl solution concentrations. It was observed how the weight of the seeds changed after 24 and 48 hours for distilled water and various concentrations of NaCl solution. Tissue paper was used to cover the Petri plates so that

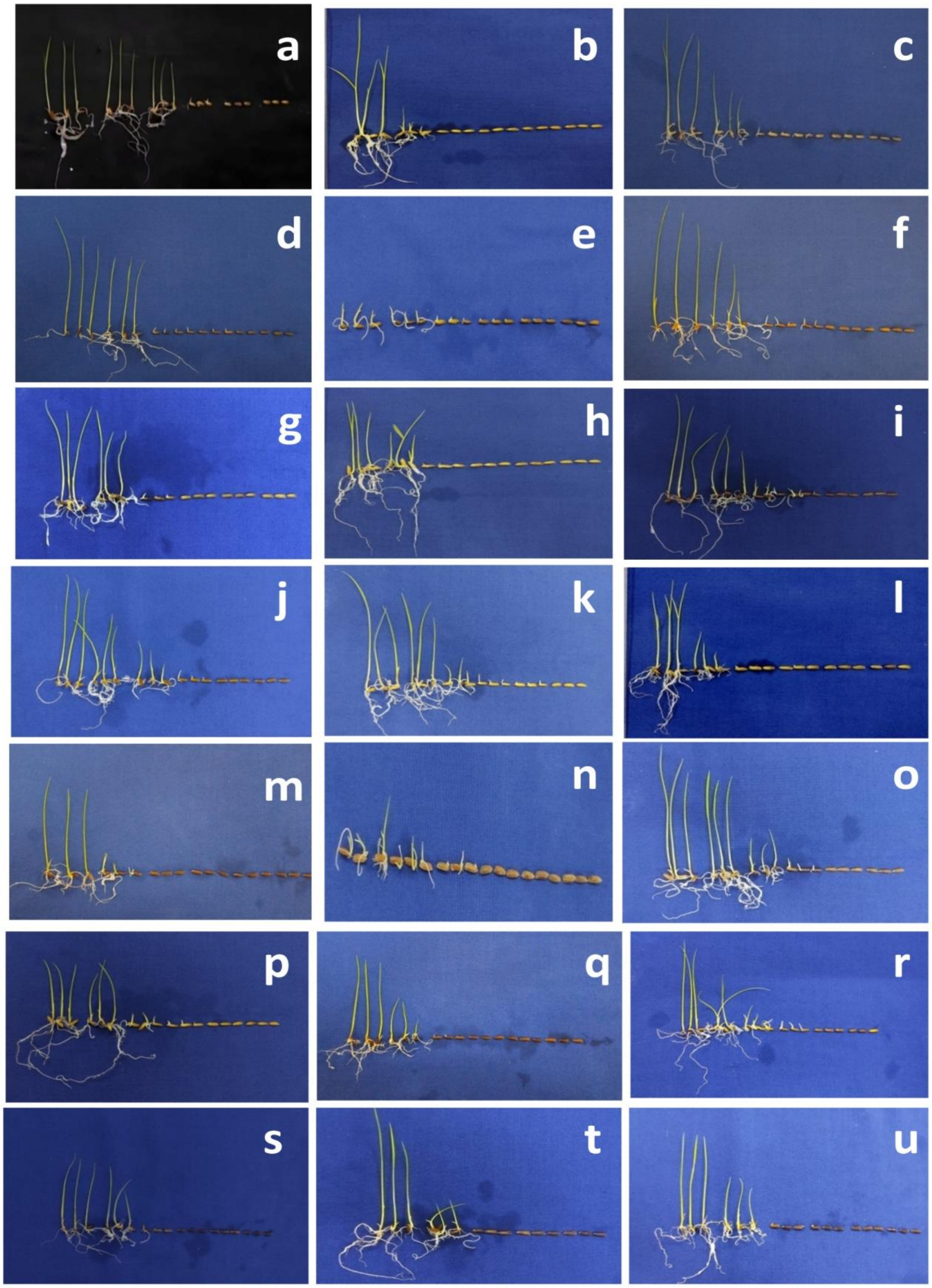

Figure 1: Seed germination under different salt concentration (control, 50mM, 100mM, 150mM, 200mM and 250mM). **a**: Yamuna, **b**:YR7, **c**:YR11, **d**:YR13, **e**:YR14, **f**:YR21, **g**:YR22, **h**:YR27, **i**:YR32, **j**:YR36, **k**:YR38, **l**:YR41, **m**:YR43, **n**:YR52, **o**:YR59, **p**:YR76, **q**:YR87, **r**:YR88, **s**:YR99, **t**:YR106 and **u**:YR109.

seeds in varying salt concentrations could germinate. To maintain the distilled water volume and the NaCl concentration, respectively, the solution was swapped out every two days. After 5 and 10 days, the rate at which the seeds germinated was measured.

Water uptake or seed imbibition, which was defined as the weight of water uptake per gram of dry seed, was calculated by weighing dry seeds and seeds that had been incubated for 24 and 48 hours. Imbibition rates were defined as the amount of imbibition water that the dry seed contained at 24 and 48 hours, respectively. Therefore, the imbibition rate (IR) (mg/g) = (W2 - W1)/W1 x 1000, where W1 (g) is the dry seed weight prior to imbibition and W2 (g) is the total seed weight (containing the weight of dry seed and imbibition water) following imbibition for 24 or 48 hours. According to Wang et al. (2011), seeds were said to have germinated when their shoot length was half of the seed length and their root length had equaled the seed length. In order to determine the overall germination percentage (GP) after five and ten days, the seeds' germination ability was monitored every day.

RESULTS

Effect of salt stress on germination and early seedling growth of rice:

Parent and breeding lines differed significantly in terms of imbibition rate, germination rate, shoot length, and root length. Over a 10-day period, the performance of seed germination at varying NaCl concentrations (0, 50, 100, 150, 200, and 250 mM) showed that germination decreased with increasing salt concentration (Table 1, Fig. 1). Both the germination % and the length of the roots and shoots were decreased by salt stress; however the germination percentage and root length were more impacted than the shoot length. Furthermore, under moderate (100 and 150 mM) saline circumstances, there was a substantial difference in root and shoot length and germination %, but not under low (0 and 50 mM) or high (200 and 250 mM) saline conditions. The breeding lines YR106 and YR43 showed highly salt sensitive in seed germination and on the other hand YR11, YR13, YR38, YR76 and YR 88 breeding lines germinate in high salt stress (200mM). Among them YR13 showed higher resistance and germinate 250mM salt concentration.

The imbibition rates at 24 h and 48 h among the Parents and breeding lines showed little variation. All the progeny showed higher imbibitions rate in 48hrs than 24 hrs and almost all breeding lines and mother parent Yamuna showed lower imbibition rate at higher salt concentration like 200mM and 250mM in comparison to other concentration.

Tolerance to salt at one developmental stage is not always the case that correlates with tolerance at later stages (Johnson *et al.,* 1996). Numerous genes regulate the salt-stress responses of plants, and environmental variation has a significant impact (Foolad *et al.*, 1997). For an accurate assessment of salt tolerance, the severity of the salt treatment—that is, the concentration, temperature, and duration of treatment—is therefore crucial. Generally speaking, separated populations for genetic studies with the greatest genotypic diversity benefit from low concentrations of salt (approximately 0.5% NaCl 86.20mM), whereas high concentrations (about 0.8% NaCl,137.93mM) are appropriate for assessing salt tolerance in germplasm (Wang *et al.*, 2004).

The intake of water by the seed and the ion toxic effect are often related to the influence of salt stress on seed germination (Levitt 1980). Since imbibition is the initial stage of seed germination, lowering the rate of imbibition might decrease imbibitional injury (Vertucci and Leopold 1987; Mccormac and Keefe 1990; Wang *et al.*, 2010). An estimate of a seed population's viability is the germination percentage. As a result, imbibition rate and germination percentage experiments were performed on seeds under salt stress and according to our findings, moderate to high salt concentrations had a significant impact on seed germination. Salt stress may prevent seeds from germinating because of their inability to absorb NaCl or because of their reduced capacity to absorb water and nutrients (Wang *et al.*, 2011). The treatment's salt concentration is very crucial for assessing seed germination under salt stress.

Table 1. Imbibition rate of parent and breeding lines in different salt concentration

Rice lines	Duration	Control	50mM	100mM	150mM	200mM	250mM
Yamuna	24 hrs	281.25	354.83	316.66	303.03	281.25	200.00
	48hrs	375.00	403.22	383.33	363.63	343.75	233.33
Wild	24 hrs	291.66	191.30	222.22	142.85	230.76	160.00
	48hrs	375.00	230.76	259.25	178.57	230.76	200.00
YR7	24 hrs	307.69	193.55	407.41	360.00	384.62	310.00
	48hrs	461.54	354.84	444.44	440.00	461.54	340.00
YR11	24 hrs	277.78	290.32	303.03	216.22	200.00	102.56
	48hrs	305.56	419.35	424.24	243.24	314.29	153.85
YR13	24 hrs	171.43	363.64	312.50	257.14	176.47	142.86
	48hrs	285.71	393.94	375.00	314.29	205.88	200.00
YR14	24 hrs	170.73	166.67	210.53	261.90	282.05	219.51
	48hrs	243.90	214.29	342.11	333.33	307.69	243.90
YR21	24hrs	218.75	200.00	269.23	241.38	166.67	250.00
	48hrs	343.75	266.67	384.62	344.83	333.33	392.86
YR22	24 hrs	218.18	230.77	346.15	178.57	148.15	259.26
	48hrs	272.73	269.23	384.62	214.29	259.26	259.26
YR27	24hrs	250.00	235.29	333.33	285.71	312.50	233.33
	48hrs	277.78	294.12	363.64	285.71	375.00	286.67
YR32	24 hrs	183.33	206.90	225.81	258.06	166.67	166.67
	48hrs	200.00	206.90	193.55	161.29	233.33	200.00
YR36	24 hrs	127.27	166.67	208.33	153.85	200.00	148.15
	48hrs	145.45	208.33	291.67	230.77	240.00	166.67
YR38	24 hrs	166.67	156.25	137.93	166.67	166.67	192.31
	48hrs	216.67	187.50	206.90	200.00	200.00	230.77
YR41	24hrs	388.89	388.89	382.35	307.69	282.05	261.11
	48hrs	444.44	416.67	411.76	333.33	307.69	316.67
YR43	24 hrs	266.67	310.34	241.38	241.38	333.33	187.50
	48hrs	300.00	413.79	344.83	379.31	407.41	218.75
YR52	24hrs	275.86	275.86	133.33	206.90	296.30	161.29
	48hrs	344.83	344.83	233.33	379.31	407.41	258.06
YR59	24 hrs	177.42	161.29	156.25	125.00	193.55	129.03
	48hrs	209.68	225.81	203.13	187.50	193.55	161.29
YR76	24 hrs	266.67	363.64	125.00	227.27	227.27	208.33
	48hrs	288.89	227.27	166.67	227.27	272.73	166.67
YR87	24 hrs	315.79	222.22	230.77	342.86	226.92	216.22
	48hrs	368.42	277.78	282.05	400.00	253.85	240.00
YR88	24 hrs	200.00	347.83	160.00	200.00	180.00	125.00
	48hrs	220.00	260.87	200.00	240.00	200.00	208.33
YR99	24 hrs	279.07	260.87	173.91	227.27	166.67	217.39
	48hrs	325.58	326.09	217.39	227.27	208.33	260.87
YR106	24 hrs	255.32	200.00	318.18	166.67	260.87	217.39
	48hrs	276.60	200.00	363.64	208.33	282.61	260.87
YR109	24 hrs	196.08	192.31	260.87	160.00	173.91	153.85
	48hrs	254.90	211.54	304.35	180.00	195.65	153.85

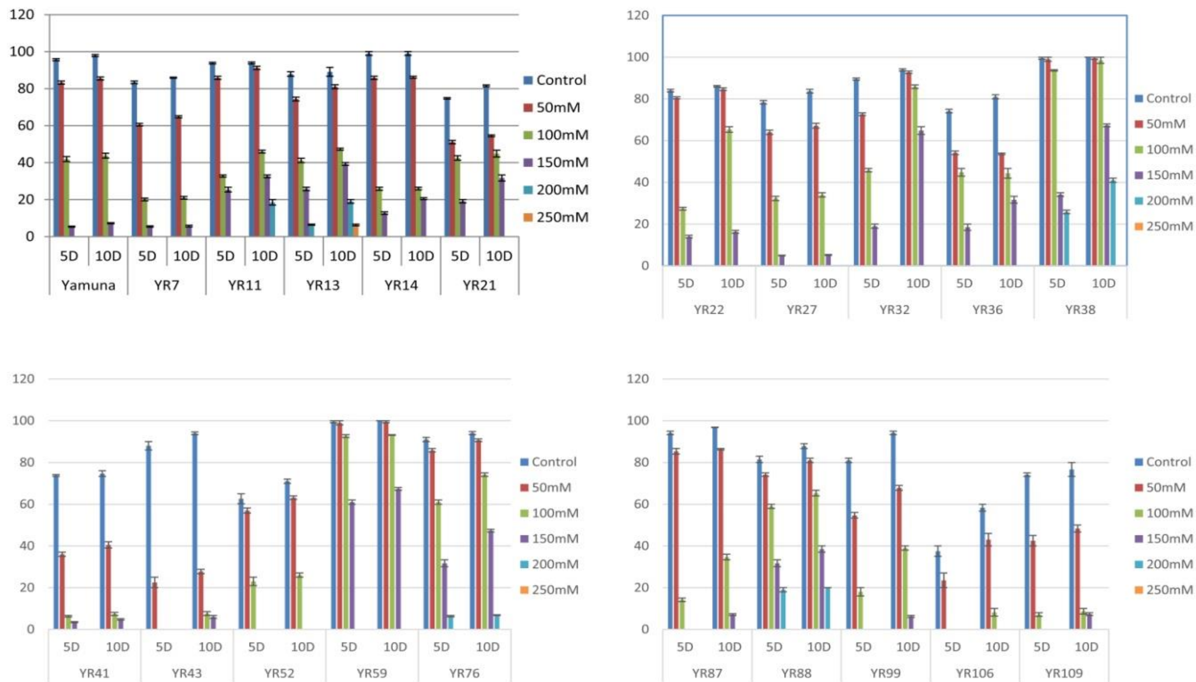

Figure 2: Germination percentage in 5 days and 10 days in salt stress

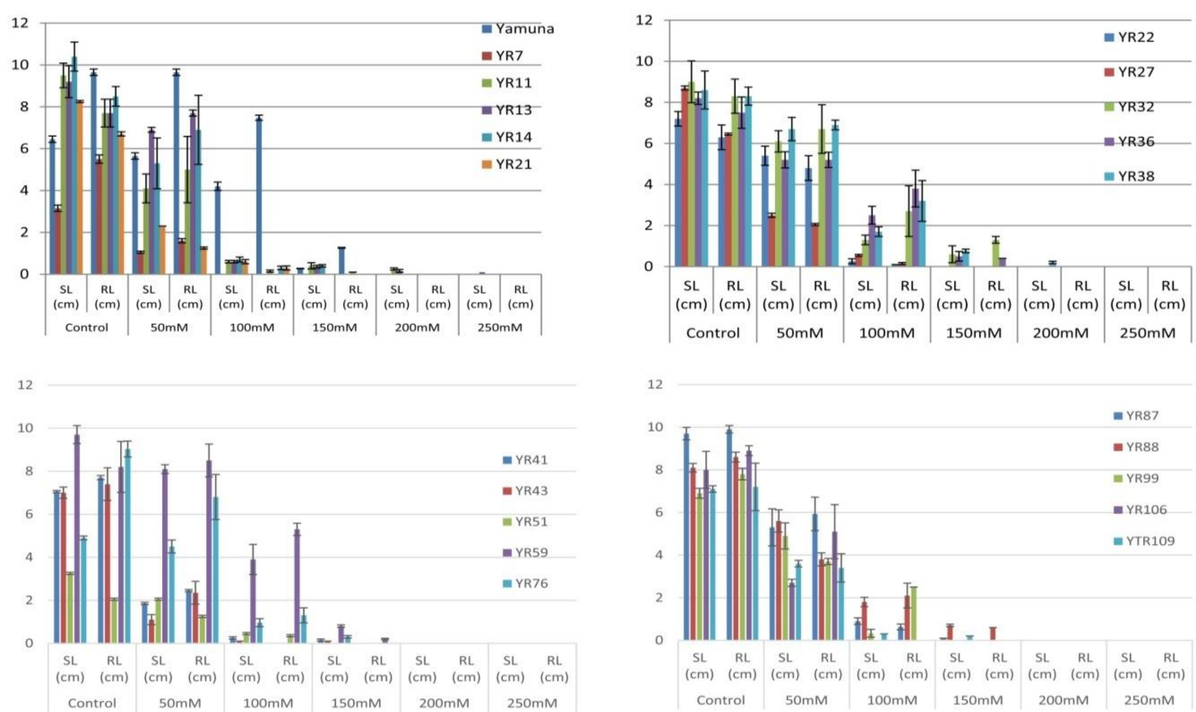

Figure 3. Root and shoot length in different salt concentration

DISCUSSION

In the growth and development of plants, seed germination is a physiologically complicated process that is very difficult to comprehend. A physical process, imbibition is the initial stage of seed germination and is mostly related to the size, structure, or chemical makeup of the seed. The size, shape, or chemical makeup of the seed may therefore represent genetic variables that regulate the rate of imbibition. Furthermore, the variability of genotypes in response to salt and intrinsic differences under normal conditions may both contribute to the performance of seed germination under salinity. Although further study is needed to determine the true genetic regulation of salt tolerance for rice seed germination, it is recommended that the existing understanding of germination capacity under salt stress will be useful in future rice breeding work.

ACKNOWLEDGEMENT

We acknowledge University of North Bengal to provide facilities for doing this research work.

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