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SALT TOLERANCE OF RICE CULTIVARS IN GREENHOUSE

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Abstract: The response of 10 rice cultivars, including the salt-tolerant variety (Pokkali) and salt-susceptible variety (IR29) as controls at five salinity levels (i.e., 0, 4, 6, 8, and 10 dS·m^{-1}) under hydroponic conditions was studied at the seedling stage in the greenhouse. The heading stage was evaluated at the salinity level of 8 dS·m^{-1} in a completely randomized block with 3 replications per each cultivar under non-saline and saline conditions. The results show that the growth of cultivars is retarded severely at the external salinity levels. Almost all leaves dried and died completely (score 7 and 9) at the level of 8 to 10 dS·m^{-1}. Cultivars IR29 and OM7347 died (score 9) at 10 dS·m^{-1}; whereas, Pokkali and IR93350 were evaluated at score 5. Salinity induces the reduction of overall agronomic parameters of cultivars, especially in dry weight relative-to-reduction over control (ROC%), salt tolerance index (STI%), pollen germination, and grain yield. Salinity-sensitive cultivars decreased their pollen viability by more than 50%, leading to a poor grain yield at a salt level of 8 dS·m^{-1}. Tested cultivars as an initial response to salinity stress were ranked as follows: OM7347, IR87832-303-1-B, and IR29 – susceptible; OM8104, IR93340, and IR93343 – moderately tolerant, and IR86385-8D-1-2-B, IR5040, IR93350, and Pokkali – tolerant.

Keywords: heading stage, pollen germination, susceptible, seedling stage, salinity-tolerant

1 Introduction

Rice is one of the most important staple crops that offer food for more than 50% of the world's population [4]. It is estimated that by the year 2025, rice production will be around 21% more than what was produced in the year 2000 [6]. Salt stress undesirably affects plant growth during all growing stages; therefore, it is a major threat to crop productivity.

Salinity stress is abiotic stress, which causes decreasing growth and productivity of glycophytes, including crops. In addition, the seedling and flowering stages are highly susceptible to salinity [18]. Saline at the seedling stage has a great influence on rice life, plant height, number of tillers, dry mass of straw and roots, root length, and growth time. During the flowering stage, salinization affects primary stems and spikelet formation, pollinating and germination of the pollen, reducing the number of effective spikelets/panicles, and increasing unfilled grain ratio [18]. Saline also causes a reduction of panicle length, grains/panicle, and 1000-grain weight, resulting in reducing productivity [1, 14]. Salt tolerance is known as the ability of the plant to survive and to complete its growth cycle under saline conditions [16]. Therefore, several reliable criteria are used for selecting the development of salt-tolerant varieties. This selection has been considered as one of the strategies to increase rice production under saline conditions. One of the highly effective solutions for rice production in saline soils is to select salinity-tolerant rice varieties suitable for each ecological region. The first important step in the selection of salt-tolerant rice varieties is screening in the greenhouse, which helps shorten the time and save the cost for the selection process before growing in the field.

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This study evaluates the difference in salt tolerance for 10 rice cultivars during the seedling and heading stages. It should provide basic data to select the salt-tolerant rice cultivars before growing in the field.

2 Materials and methods

Materials

Ten rice cultivars, namely OM7347, OM8104, IR93340, IR93343, IR5040, IR93350, IR87832-303-1-B, IR86385-8D-1-2-B, IR29, and Pokkali were acquired from Cuu Long Rice Research Institute and the International Rice Research Institute (IRRI). The salt-sensitive rice variety IR29 from IRRI and the salt-tolerant rice variety Pokkali from the National Institute of Agrobiological Sciences (NIAS) Genebank (Japan) were used as controls.

Methods

Experiment 1

A net-house experiment at the seedling stage was conducted with five treatments at different salt concentrations (electrical conductivity EC = 0, 4, 6, 8, and 10 $dS{\cdot}m^{-1}$) under hydroponic conditions. Each concentration was arranged for rice varieties in sequential order. The screening on salinity tolerance in the seedling stage was accomplished according to an IRRI's protocol in the Yoshida medium [21] by adding NaCl of different concentrations.

Monitoring indicators include

i. Root length (cm): After three weeks of planting, the root length was measured with 10 plants for each concentration.

ii. Fresh weight and dry weight of the plant (g): The shoots and roots of plants for each concentration were weighed fresh and then dried at 70 °C until constant weight.

iii. Dry weight reduction over control (ROC): The inhibition effect on seedling dry weight induced by salinity was calculated as the percentage reduction over control (% ROC) according to [2].

$$\%\,ROC = \frac{\text{Value in control} - \text{Value in saline environment}}{\text{Value in control}} \times 100$$

iv. Salt tolerance index (STI): STI was calculated as the total dry weight of plants obtained from different salt concentrations compared with the total plant dry weight obtained from the control.

$$STI = (TDW_{\text{at Sx}}/TDW_{\text{at S1}}) \times 100$$

where TDW is the total dry weight; Sx is the salt level treatment; S1 is the control treatment [16].

The classification of salinity tolerance is as follows: 0–20% reduction (tolerant – T); 21–41% reduction (moderately tolerant – MT); 42–60% reduction (moderately susceptible – MS); >60% reduction (susceptible – S) [8].

The salinity tolerance was evaluated according to the standard evaluation score (SES) of visual salt injury at the seedling stage as follows [11]:

i. Score 1: Normal growth – there are no leaf symptoms (highly tolerant);
ii. Score 3: Nearly normal growth – leaf tips or few leaves are whitish and rolled (tolerant);
iii. Score 5: Growth severely retarded – most leaves are rolled; only a few are elongating (moderately tolerance);
iv. Score 7: Complete cessation of growth – most leaves are dry; some plants are dying (susceptible);
v. Score 9: Almost all plants died or are dying (highly susceptible).

The monitoring and evaluation of these indicators were conducted on the 21st day after the saline treatment.

Experiment 2

A completely randomized block design with three replications was applied to the second experiment in the net-house. Fifteen rice plants with 3–4 leaves from Experiment 1 were chosen from the treatment with concentration 0 dS·m^{-1} for each cultivar. They were cultivated in water without salt from the seedling stage until the panicle initiation. When the first flag leaf appeared, the rice crops were transferred in the saline medium (kept at EC = 8 dS·m^{-1} and pH = 5 ± 0.5, and checked at 8–10 a.m. every day and maintained consecutively for 10 days). After this period, the appearance of the panicle in the flag leaf cover was checked.

For measurements of pollen viability, six samples of unopened spikelets at the reproductive stage were randomly collected with three replications and stored in 70% ethanol in the refrigerator for analysis. In the analysis, the anthers from the stored spikelet were removed and crushed thoroughly to release all the pollens. Pollens were stained with a drop of a 1% potassium iodide (KI) solution. The samples were covered with the coverslip and viewed under a light microscope at 20× magnification. Fertile and unfertile pollens were counted at three different areas per slide. Pollen viability was calculated by dividing the number of fertile pollen grains to the total number of pollen grains and expressed in percentage.

Data analysis: The comparison of the differences of indicators between experimental varieties with one-way analysis of variance was performed on Statistix 10.0, and average means ± SD were calculated.

3 Results and discussion

3.1 Screening at early seedling stage

The results on 10 rice cultivars after 21 days in the salinized solution at different salinity levels are presented in Table 1. The survival time of plants is a significant parameter under the saline condition. The salt-sensitive varieties could not grow in the presence of high salt levels, and their growth was inhibited by osmotic stress and toxic ions (Na^+, Cl^-), which ultimately caused plant death. Such growth inhibition might even occur with short-term salt stress. The data show that rice seedlings of OM7347 and IR29 died completely at EC = 10 dS·m^{-1} and survived only 15 and 10 days, respectively, and other cultivars survived 21 days. Depending on leaf rolling and leaf drying characteristic performance under salt stress at the salinity concentration of 4 dS·m^{-1}, OM8104, IR93343, IR93350, IR87832-303-1-B, and Pokkali remained developed, but the others (e.g. OM7347, IR93340, IR5040, IR86385-8D-1-2-B, and IR29) suffered from leave rolling (score 3). When EC increased to 6 dS·m^{-1}, all the varieties were injured with leaf rolling and leaf drying on the tip (score 3). Specifically, IR29 stopped its development completely; almost all of the leaves were dry; some of the shoots died (score 7).

Table 1. Survival time and evaluation of tolerant salinity after 21 days in a salinized solution

Cultivar	Survival time (day)					Evaluation of tolerant salinity (score)				
	Electrical conductivity (dS·m^{-1})									
	0	4	6	8	10	0	4	6	8	10
OM7347	21	21	21	18	15	1	3	5	7	9
OM8104	21	21	21	21	21	1	1	3	5	7
IR93340	21	21	21	21	21	1	3	3	5	7
IR93343	21	21	21	21	21	1	1	3	5	7
IR5040	21	21	21	21	21	1	3	3	5	7
IR93350	21	21	21	21	21	1	1	3	5	5
IR87832-303-1-B	21	21	21	21	21	1	1	3	5	7
IR86385-8D-1-2-B	21	21	21	21	21	1	3	3	5	5
IR29 (sensitive)	21	21	21	10	10	1	3	7	9	9
Pokkali (tolerant)	21	21	21	21	21	1	1	3	3	5

The sensitive variety IR29 died completely; 45% of shoots of OM7347 also died at 8 dS·m^{-1}. The other 7 cultivars had a saline tolerance at score 5, except the control Pokkali (score 3). Other cultivars suffered from shoot death and cessation of growth (score 7), except IR93350, IR86385-8D-1-2-B (score 5), and Pokkali at 10 dS·m^{-1} (Table 1). Osmotic stress, which is created by the accumulation of ions at the rhizosphere, is the first effect of salt stress that limits the water extraction ability of the roots and eventually leads to plant growth reduction. Ionic disequilibrium is the second effect of salt excess that quickly overtakes osmotic stress in rice and leads to nutrient starvation, enzyme inactivation, oxidative stress, and ionic toxicity in plant tissues [15, 20].

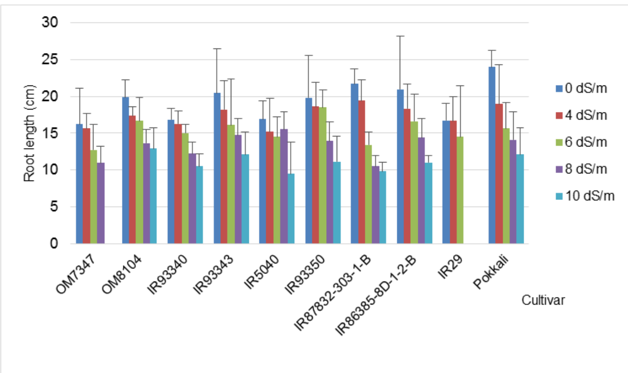

Figure 1. Effect of salinity levels on root length of different rice cultivars
(After 21 days in salinized solution, data means ± SD, n = 10)

Measurements were made on 3-week old hydroponically grown plants after exposure to salt stress. The root length of all the rice cultivars declined with an increase in salinity in all the salt treatments compared with the control (Figure 1). The root length of OM7347 (10.98 cm) is the smallest at EC = 8 dS·m^{-1}, and the cultivar died at EC = 10 dS·m^{-1}, which is similar to salt-sensitive IR29 (100% dead). At the salinity level up to 10 dS·m^{-1}, OM8104 and IR93343 showed better performance, followed by Pokkali. Although varieties OM8104, IR93343, and Pokkali had produced significantly larger root length at this salt level compared with other varieties, the root length is only around 50% of that of the control. Therefore, the gradual decrease in root length with the increase of salinity might be due to the more inhibitory effect of NaCl to root growth compared with that of shoot growth.

As a result, the root length and dry matter weight significantly reduced with the external salinity levels as well as the performance, including leaf rolling and leaf drying at the seedling stage (Table 1, 2, and Figure 1). A higher concentration of NaCl shows strong inhibition effects compared with lower concentrations. The reduction in seedling fresh and dry weight is due to decreasing water uptake by seedlings in salt presence. In previous studies, the authors reported that salt stress causes the reduction of water uptake of roots or water loss from roots, depending on the level of the stress, which seriously decreases plant growth [7, 10, 13].

About 80–90% dry matter weight that plants store is the product of photosynthesis, and the rest is the absorbed minerals nutrition from soil [3]. Under the salinity-induced conditions, leaf rolling and leaf drying are morphological indicators related to salt tolerance of rice, leading to reducing fresh and dry matter weight. The dry matter weight of all the rice cultivars declined with increasing salt concentrations (Table 2). Different cultivars were pronounced in this character. OM7347 died at a salt concentration of 10 dS·m^{-1}. The lowest dry weight is observed with IR5040 at 0.04 g·plant^{-1} (10 dS·m^{-1}). The salt-sensitive variety IR29 died at 8 dS·m^{-1}. At all salinity levels, Pokkali had the highest dry weight, much higher than other cultivars, and its reduction of dry weight is not significant. It has been reported that increasing salinity at the seedling stage often causes osmotic effect that leads to decreasing the rate of water uptake and/ or specific ion toxicity, and as a result, the root length and root weight may reduce, and leaf rolling and drying increases due to reducing dry matter weight [9]. Thanks to the cell-to-cell water movement via the expression of an active water channel, the overall flow of water from the root-shoot-leaf continuum increases, and, therefore, the evapotranspiration through stomata is maximized.

Table 2. Effect of salinity levels on dry matter weight and salt-tolerant index of 10 rice cultivars

Cultivar	Dry matter weight (g·plant^{-1})					Salt tolerant index STI (%)			
	Electrical conductivity (dS·m^{-1})								
	0	4	6	8	10	4	6	8	10
OM7347	0.32	0.23	0.15	0.14	–	71.88	46.88	43.75	–
OM8104	0.31	0.28	0.21	0.20	0.18	90.32	67.74	64.52	58.06
IR93340	0.19	0.16	0.13	0.13	0.10	84.21	68.42	68.42	52.63
IR93343	0.18	0.16	0.16	0.15	0.10	88.89	88.89	83.33	72.22
IR5040	0.16	0.16	0.10	0.08	0.04	100.00	62.50	50.00	25.00
IR 93350	0.19	0.17	0.17	0.15	0.12	89.47	89.47	78.95	63.16
IR87832-303-1-B	0.25	0.22	0.21	0.18	0.15	88.00	84.00	72.00	60.00
IR86385-8D-1-2-B	0.39	0.25	0.21	0.19	0.16	64.10	53.85	48.72	41.03
IR29 (sensitive)	0.06	0.05	0.04	–	–	83.33	66.67	–	–
Pokkali (tolerant)	0.54	0.51	0.49	0.49	0.47	94.44	90.74	90.74	87.04

Note: (–) the plant died completely. Dry matter weight from 21-day old seedlings.

From the current results, it seems that the effect of NaCl on the salt tolerance index of cultivars is significant (Table 2). The salt tolerance index decreases under high salt concentrations. The salt tolerance index ranges widely from 100.00% at 4 dS·m^{-1} of NaCl to 25.00% at 10 dS·m^{-1} of NaCl, a significant 4-fold decrease observed in the IR5040 cultivar; meanwhile, IR29 and OM7347 could not grow at 8 to 10 dS.m^{-1}, respectively. In general, high salt concentration results in the reduction of plant growth, leaf expansion, root length, and root weight. Accordingly, it leads to low dry matter weight. Therefore, the salt-sensitive varieties had a lower salt-tolerant index than the salt-tolerant varieties (IR93343, IR93350, IR86385-8D-1-2-B, and Pokkali) within the 21-days trial.

Table 3. Effect of salinity levels on reduction over control (ROC%) and salinity tolerance of rice cultivars

Cultivar	Reduction over control ROC%				Salinity tolerance			
	Electrical conductivity (dS·m^{-1})							
	4	6	8	10	4	6	8	10
OM7347	25.00	56.25	73.91	–	MT	MS	S	S
OM8104	22.58	35.71	35.48	41.90	MT	MT	MT	MS
IR93340	15.79	37.50	31.58	47.40	T	MT	MT	MS
IR93343	11.11	12.50	16.67	27.80	T	T	MT	MS
IR5040	37.50	37.50	50.00	75.00	MT	MT	MS	S
IR 93350	10.53	11.76	21.05	36.80	T	T	MT	MT
IR87832-303-1-B	4.00	18.18	28.00	40.00	T	T	MT	MT
IR86385-8D-1-2-B	10.26	35.90	51.28	59.00	T	MT	MS	MS
IR29 (sensitive)	33.33	33.33	–	–	MT	MT	S	S
Pokkali (tolerant)	3.70	5.56	9.26	13.00	T	T	T	T

Note: T is tolerant; MT is moderately tolerant; MS is moderately susceptible; S is susceptible. The percentage of reduction over control (% ROC) was calculated as the inhibition effect on seedling dry weight induced by salinity when compared with the control.

The reduction-over-control results (Table 3) show that when the level of NaCl increases, the reduction in dry weight also increases. The 10 dS·m^{-1} of NaCl had the highest reduction (75.00%) in IR5040, followed by IR86385-8D-1-2-B with 59.00%. At 8 dS·m^{-1} of NaCl, the reduction is 73.91% in OM7347, and IR29 (susceptible) died completely. According to ROC%, the salinity tolerance of the rice cultivars decreased when the salt concentration increased from 4 dS·m^{-1} to 10 dS·m^{-1}. At 4 dS·m^{-1}, the 10 rice cultivars were tolerant and moderately tolerant. When the salt level increased to 6 dS·m^{-1}, OM7347 became moderately susceptible. From 8 to 10 dS·m^{-1}, the OM7347 and IR29 cultivars were susceptible, and the seedlings died completely. At 8 dS·m^{-1}, IR5040 and IR86385-8D-1-2-B were moderately susceptible; whereas, other cultivars were moderately tolerant. However, when the salinity level increased to 8 and 10 dS·m^{-1}, all the cultivars showed gradual deviation from their previous salt-tolerant ranking from T to MT, MT to MS, and MS to S, respectively. In this study, at the salinity of 10 dS·m^{-1}, nearly all cultivars became susceptible to salt, except IR93350, IR87832-303-1-B (moderately tolerant), and Pokkali (tolerant).

3.2 Screening at the heading stage and grain yield

The reproductive stage includes the process of pollination and fertilization to form grains [12, 17]. Therefore, it is one of the most sensitive growth stages regarding the saline condition. Experiment 2 was conducted at the booting/heading stage for 20 days from the day of "flag leaf appearance" to define the fertilization rate under saline conditions at 8 dS·m^{-1}, which provides the assessment foundation for the effect of saline on pollen germination, number of unfilled spikelets, number of filled spikelets, and grain yield in both water without salt (control) and salinized conditions (saline).

Table 4. Effect of saline on pollen germination and grain yield

Cultivar	Pollen germination (%)		% decrease	Salinity tolerance	Grain yield per plant (g·plant^{-1})	
	Control	Saline			Control	Saline
OM7347	85.16^{cd}	53.11^{ef}	32.05	S	4.50^{bc}	1.32^{de}
OM8104	90.31^{bc}	69.73^{bcde}	20.58	MT	6.20^{ab}	3.49^{bc}
IR93340	84.65^{d}	61.41^{def}	23.24	MT	5.38^{bc}	2.39^{cd}
IR93343	90.60^{b}	64.59^{cde}	26.01	MT	4.63^{bc}	2.51^{cd}
IR5040	96.38^{a}	91.26^{a}	5.12	T	6.01^{ab}	4.69^{ab}
IR93350	92.48^{ab}	83.89^{abc}	8.59	T	5.38^{bc}	3.29^{c}
IR87832-303-1-B	93.06^{ab}	40.68^{fg}	52.38	S	4.99^{bc}	1.11^{e}
IR86385-8D-1-2-B	91.96^{ab}	82.90^{abcd}	9.06	T	7.33^{a}	5.02^{a}
IR29 (sensitive)	81.63^{d}	30.56^{g}	51.07	S	4.03^{c}	0.70^{e}
Pokkali (tolerant)	94.30^{ab}	87.83^{ab}	6.47	T	7.56^{a}	5.23^{a}
F test	1.41	0.07	–	–	0.02	14.90
CV%	3.49	17.87	–	–	19.26	24.89
$LSD_{0.05}$	5.40	21.48	–	–	1.85	1.27

Note: Values in the same column with the same letter indicate no significant difference at α = 0.05.

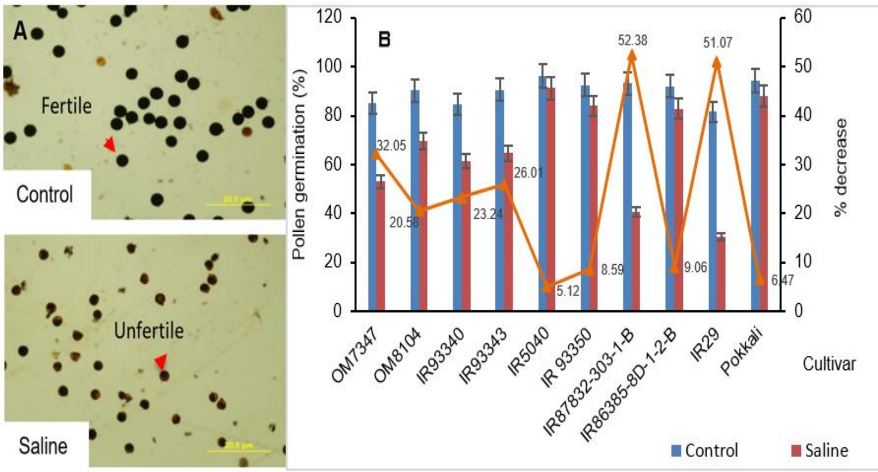

Figure 2. Effect of salinity at EC = 8 dS·m^{-1} on pollen germination of ten rice cultivars. A) Observation of pollen viability at EC = 8 dS·m^{-1} under a light microscope at 20× to illustrate pollen fertile in control and unfertile in saline conditions. B) The percentage of pollen germination in saline and non-saline (control) conditions. The data are shown as the means ± SD with 3 replications.

Under saline conditions (EC = 8 $dS{\cdot}m^{-1}$) within 10 days, until the panicle appears, the pollen germination clearly decreases in comparison with the non-stress condition. All the cultivars show inconsistency in salt tolerance over increasing salt concentration. In which, Pokkali and IR5040 had the highest pollen viability in both environmental conditions, and 4/10 cultivars were salt-tolerant, namely IR5040, IR93350, IR86385-8D-1-2-B, and Pokkali. Whereas, with IR29 (a sensitive variety), the pollen fertilization rate under the saline conditions decreased to nearly a half (by 51.07%) in comparison with the non-saline conditions. Similarly, the pollen fertilization rate of IR87832-303-1-B declined by 52.38% and OM7347 by 32.05%, and they were classified as susceptible. Three over ten cultivars were moderately tolerant (Table 4, Figure 2).

High concentrations of salts in the soil increase osmotic pressure and reduce water potential, which eventually causes a reduction in water uptake or loss of water. Almost all of the cultivars decreased pollen viability owing to salinity, leading to poor grain yield: OM7347 (1.32 $g{\cdot}plant^{-1}$), IR87832-303-1-B (1.11 $g{\cdot}plant^{-1}$), and IR29 (0.70 $g{\cdot}plant^{-1}$) at EC = 8 $dS{\cdot}m^{-1}$. In contrast, Pokkali had the highest grain yield in both control and saline conditions with 7.56 and 5.23 $g.plant^{-1}$, respectively. IR86385-8D-1-2-B and IR5040 had a grain yield of 5.02 $g{\cdot}plant^{-1}$ and 4.69 $g{\cdot}plant^{-1}$, respectively, and it is not statistically significantly different from that of Pokkali. Additionally, previous studies demonstrated that germination, active tillering, and maturity stages are considered to be less sensitive to salinity than the seedling stage, early reproductive stage, pollination, and seed formation [5, 17]. Indeed, under saline conditions, a high concentration of Na^{+} and Cl^{-} provides toxic ions to all plant cells. Specifically, the heading stage of rice is one of the most sensitive stages of salt stress. On the other hand, a reduction in the growth of rice from germination to maturity may be due to the increase of osmotic pressure in the root cells and/or by the specific ion effects or both.

Moreover, the results clearly show that IR5040 is considered as a sensitive variety at the seedling stage; whereas, it had been found to be tolerant at the heading stage at 8 $dS{\cdot}m^{-1}$. This result agrees with Singh & Flowers' findings [19], which states that rice is the most sensitive to salinity at two growth stages, but, unfortunately, they are independent of each other and controlled by different sets of genes. A genotype has good salinity tolerance at the seedling stage, which does not ensure that such tolerance would remain the same at the reproductive stage. In addition, the expression and regulation of these genes at altered stages are different. This leads to the plant's different performances in phenotypes. Therefore, it is necessary to understand the genetic and physiological control mechanisms of salt tolerance to facilitate the development of new varieties under high salt levels.

4 Conclusions

It is obvious that the increasing levels of NaCl reduces the growth of rice plants, expressed through the morphological characteristics: leaf rolling, tip leaf drying, plant height, root length, and biomass of the seedling rice stage. Depending on the different tolerance, the rice cultivars have a longer or shorter survival time at different levels of salinity. The seedlings die if the salinity is too high, especially discontinuing growing at 8 $dS{\cdot}m^{-1}$ or more. Salinity stress has a severe effect on the heading stage, causing pollen inviability, decreasing the germination rate, leading to a decrease in the rice grain yield. Salinity-sensitive cultivars whose pollen germination rate decreases by 30–50% and >50% have a loss of yield of >50% if salinity is too severe. The salt tolerance of the cultivars at EC = 8 $dS{\cdot}m^{-1}$ is as follows: OM7347, IR87832-303-1-B, and IR29 – susceptible;

OM8104, IR93340, and IR93343 – moderately tolerant; IR86385-8D-1-2-B, IR5040, IR93350, and Pokkali – tolerant. The salinity of 8 $dS \cdot m^{-1}$ is the threshold tolerance for rice cultivars in the experiment.

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