Testing Method for Salinity Tolerance at Germination Stage on Rice Genotypes

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Abstract

Salinity is a major constrain to high yield of rice (Oryza sativa L.) grown in coastal area. Testing method for salinity tolerance is performed in a greenhouse by transplanting 14 days-old seedling on soil salinized with 4000 ppm NaCl. The study to develop a testing method for salinity tolerance at germination stage was conducted from September 2016 to March 2017. The germination test with rolled paper towel test treated with 4000 ppm NaCl was identified as the best to differentiate tolerant and susceptible genotypes. The early and green house methods were applied to evaluate the salt tolerance of 40 genotypes. Two weeks old rice seedlings were transplanted into plastic tubs filled with 5 kg of dried soil watered with 4 l of 4000 ppm NaCl solution. The experiment was performed in a RCD with three replications for the green house and a RCBD with three replications for early method. The testing method at germination as well as the vegetative stage could classify the rice genotypes into three groups, tolerant, moderately tolerant, and susceptible. Salinity tolerance at germination stage was closely correlated to those at vegetative stage with 0.71correlation coefficient.

Keyword: breeding lines, coastal area, development of method, NaCl, screening.

Introduction

Rice is a crop that is sensitive to salinity. Salinity on rice can decrease seed germination (Parveen et al., 2016), interfere the growth and survival of seedlings (Pattanagul and Thitisaksakul 2008), damage the chloroplast structure so that decrease chlorophyll content (Zhang et al., 2012), decrease photosynthesis capacity (Moradi and Ismail 2007), decrease grain filling and yield (Joseph and Mohanan 2013). Rice productivity in saline areas is enhanced by planting salinity tolerant rice. The salinity of rice is determined by time and money consuming selection at vegetative stage. Selection of rice genotypes should be conducted as early as possible within a short period with relatively low cost. Top paper test method using filter paper moistened with NaCl solution of 5 dSm-1 (Pradheeban et al., 2014), 10 dSm-1 (Ologundudu et al., 2014), 15 dSm-1 (Islam and Karim 2010), 100 mM (Mokhtar et al., 2015), 14% (Kandil et al., 2012), and top paper test method using blotter paper moistened with 30 mM - 150 mM NaCl solution (Abbas et al., 2013) can be used to test the rice genotype salinity tolerance level at the germination stage in the laboratory.

Current testing for rice tolerance at germination stage is widely various. The existing test methods have not yet provided the information about their relation with the testing methods that have been used. Therefore, a rapid, accurate salinity tolerance testing methods at the germination stage which has a close correlation with the vegetative stage is indispensable. The objective of this study is to obtain an appropriate testing method for rice salinity tolerance at germination stage which can be used for rice genotypes selection.

Materials And Methods

Identification of Testing Methods for Salinity Tolerance at Germination Stage

The identification of potential methods to test the tolerance to salinity of rice at the germination stage was carried out through germination test using two methods of Rolled Paper Towel Test and Top Paper Test using stencil paper treated with salinity. The experiment was conducted using a randomized completely block design (RCBD) with three replications. The treatments used were 2 genotypes, tolerant (Pokkali and Inpari 34) and susceptible (IR 29 and Pelopor 2193), and NaCl concentration consisting 6 levels i.e. 0, 2000, 4000, 6000, 8000, and 10000 ppm.

Rolled Paper Towel Test

Fifty seeds were germinated in the rolled paper towel test method using stencil paper with prescribed salinity. The vigor index was observed on fifth day, germination percentage on fifth day and seventh day, and maximum growth potential on seventh day. Normal seedling criteria were observed based on ISTA (2014). The seedling dry weight was observed by using oven method at 60 °C for 72 hours. Plumule and radicle length was measured at seventh day. root length relativ was determined by Rx / Ro formula, where the plumule length was at x ppm (Rx) and radicle length at 0 ppm (R0). The data collected were analyzed using *Least Significant Different* (LSD) test.

Top Paper Test

Fifty seeds were germinated in the top paper test method using a plastic box (14 cm x 9 cm x 6 cm) wrapped by stencil paper with prescribed salinity. The observation on the vigor index, germination, maximum growth potential, radicle and plumule length, relative root length, and decreased radicle and plumule length were perfumed. The data collected were analyzed using *Least Significant Different* (LSD) test.

Rice Breeding Lines Tolerance Salinity Test

The tested rice genotype involved 36 breeding lines obtained from Indonesian Centre of Rice Research (ICRR), 2 tolerant genotypes and 2 susceptible genotypes to salinity. The salinity tolerance test was conducted at germination and vegetative stage. The test at the germination stage was using a method identified in the previous experiment, that was rolled paper towel test 4000 ppm of NaCl solution. The experiment used a randomized complete block design (RCBD) with three replicates, with variables of vigor index, germination percentage, and radicle length.

The test at vegetative stage used the methods performed on rice breeding. The experiment used a randomized complete design (RCD) with three replicates. 14 days-old rice seedlings were transferred into plastic tubs (40 x 30 x 11 cm) containing 5 kg of latosol. Media salinity was given by adding 4 liters of 4000 ppm NaCl solution and left for 24 hours before planting (Sulaiman 1980). Each tub was planted by 4 breeding lines of rice; each breeding line was planted with 3 seedlings at 14 days-old after seedling then grown to 6 weeks after planting. The water level of puddle was maintained by adding water every day. Percentage of dead leaves of each breeding line was observed at 14 days after transplanting based on Sulaiman 1980 method.

Results and Discussion

Identification of Testing Method for Salinity Tolerance at Germination Stage

Rolled Paper Towel Test

The vigor index, germination percentage and maximum growth potential of rice in rolled paper towel test method with different salinity are presented in Table 1. Vigor index and germination percentage of rice showed different tolerances at salinity concentration of 0, 2000, 4000, and 6000 ppm. The viability at 0 ppm was different due to the genetic differences. Each lot of seeds according to Belo and Suwarno (2012) had a different viability due to the genetic factors. Differences in the vigor index and germination percentage of salinity at 2000, 4000, and 6000 ppm were estimated due to the salinity osmotic stress which would suppress the rice germination.

Table 1Differences ofthe value of (delta) vigor index, germination percentage, and maximum growth potential between salinity of tolerant and susceptible rice genotypes on rolled paper towel test and top paper test methods and different concentrations of NaCl

NaCl Concentration	Rolled paper towel test			Top paper test		
	Tolerant genotypes	Susceptible genotypes	Delta	Tolerant Genotypes	Susceptible genotypes	Delta
Vigor index(%)						
0	80.37	62.23	18.14*	67.33	68.00	-0.67
2000	75.56	54.08	21.48*	52.00	57.33	- 5.33*
4000	50.38	27.41	22.97*	39.44	31.67	7.77*
6000	27.41	13.60	13.81*	5.67	2.33	3.34
8000	0	1.11	-1.11	0	0	0
10000	0	0.74	-0.74	0	0	0
Germination percentage(%)						
0	89.63	94.45	-4.82	92.67	91.67	1.00
2000	89.26	68.89	20.37*	87.00	86.33	0.67
4000	73.83	47.41	26.42*	73.22	70.33	2.89
6000	51.12	31.11	20.01*	37.00	34.33	2.67
8000	33.71	13.71	20.00*	15.67	4.67	11.00
10000	15.19	2.96	12.23	2.67	0	2.67
Maximum growth potential (%)						
0	99.63	99.63	0	100	97.00	3.00
2000	99.63	99.63	0	99.33	98.33	1.00
4000	88.89	99.26	-10.37	87.56	98.00	-10.44
6000	100.00	99.63	0.37	99.67	98.67	1.00
8000	100.00	99.26	0.74	99.00	97.00	2.00
10000	100.00	100.00	0	99.67	94.33	5.34

Note: * Significant based on LSD test at $\alpha = 0.05$ using STAR software.

Table 2 Plumule and radicle length of rice genotypes on rolled paper towel test and top paper test method

NaCl Concentration (ppm)	Rolled paper towel test			Top paper test		
	Tolerant Genotypes	Susceptible Genotypes	Delta	Tolerant Genotypes	Susceptible genotypes	Delta
Length of seedling plumule (cm)						
0	5.08	5.20	-0.12	3.52	3.99	-0.47
2000	4.61	5.16	-0.55	3.45	4.03	-0.58
4000	3.07	3.44	-0.37	2.46	2.81	-0.35
6000	3.76	4.13	-0.37	2.21	2.10	0.11
8000	2.20	1.95	0.25	1.22	1.39	-0.17
10000	0.94	1.15	-0.21	0.65	0.77	-0.12
Length of normal seedling plumule(cm)						
0	7.37	7.19	0.18	4.32	4.18	0.14
2000	6.31	7.88	-1.57	3.77	4.45	-0.68
4000	5.87	5.25	0.62	2.89	3.46	-0.57
6000	4.87	5.44	-0.57	2.79	2.90	-0.11
8000	3.72	3.18	0.54	1.89	2.15	-0.26
10000	0.38	1.67	-1.29	0.86	0	0.86
Length of seedling radicle (cm)						
0	4.71	4.52	0.19	6.15	5.17	0.98
2000	4.41	3.80	0.61	5.34	4.72	0.62
4000	3.54	2.57	0.97	5.02	3.96	1.06
6000	3.04	2.44	0.60	4.96	3.15	1.81
8000	2.89	2.36	0.53	4.37	3.14	1.23
10000	1.63	1.79	-0.16	3.28	2.18	1.10
Length of normal seedling radicle (cm)						
0	7.49	8.02	-0.53	6.45	5.43	1.02

2000	7.45	8.13	-0.68	5.88	5.01	0.87
4000	6.66	6.57	0.09	5.71	4.57	1.14
6000	8.69	9.20	-0.51	5.66	4.08	1.58
8000	8.80	6.88	1.92	5.29	3.72	1.57
10000	1.23	3.78	-2.55	1.94	0	1.94

Note: Not significantly based on LSD test at α = 0.05 using STAR software.

Table 3 Decrease of plumule and radicle length of rice genotype on rolled paper towel test and top paper test method

NaCl Concentration (ppm)	Rolled paper towel test			Top paper test		
	Tolerant Genotypes	Susceptible Genotypes	Delta	Tolerant Genotypes	Susceptible genotypes	Delta
Decrease length of seedling plumule (cm)						
0	0	0	0	0	0	0
2000	11.05	-0.48	11.53	-2.27	-1.23	-1.04
4000	28.08	30.40	-2.32	16.45	29.87	-13.42
6000	27.04	17.87	9.17	33.21	47.95	-14.74
8000	57.42	61.19	-3.77	63.06	65.28	-2.22
10000	80.72	77.12	3.60	79.62	80.80	-1.18
Decrease length of normal seedling plumule (cm)						
0	0	0	0	0	0	0
2000	11.27	-14.95	26.22	1.82	-7.78	9.60
4000	8.17	23.49	-15.32	11.27	16.61	-5.34
6000	32.88	19.89	12.99	25.08	30.00	-4.92
8000	46.50	51.56	-5.06	49.94	48.27	1.67
10000	96.25	72.85	23.40	71.92	100.00	-28.08
Decrease length of seedling radicle (cm)						
0	0	0	0	0	0	0
2000	0.07	15.20	-15.13	10.97	7.85	3.12
4000	10.02	40.86	-30.84	5.19	22.49	-17.30
6000	30.18	43.90	-13.72	15.27	37.32	-22.05
8000	33.32	45.70	-12.38	24.71	37.45	-12.74
10000	59.89	59.00	0.89	44.22	56.56	-12.34
Decrease length of normal seedling radicle (cm)						
0	0	0	0	0	0	0
2000	0.30	-7.49	7.79	7.11	7.1	0.01
4000	0.60	11.44	-10.84	-1.77	14.71	-16.48
6000	-14.34	-23.53	9.19	9.06	22.31	-13.25
8000	-16.41	13.38	-29.79	15.10	30.24	-15.14
10000	85.98	41.24	44.74	65.25	100	-34.75

Note: Not significantly based on LSD test at α = 0.05 using STAR software.

Table 4 Relative root length and seedling dry weight of rice genotype on rolled paper towel test and top paper test methods

NaCl Concentration (ppm)	Rolled paper towel test			Top paper test		
	Tolerant genotypes	Susceptible Genotypes	Delta	Tolerant Genotypes	Susceptible genotypes	Delta
Relative length of seedling root						
0	1	1	0	1	1	0
2000	0.99	0.85	0.14	0.89	0.92	-0.03
4000	0.79	0.59	0.20	0.84	0.77	0.07
6000	0.7	0.56	0.14	0.85	0.63	0.22
8000	0.67	0.54	0.13	0.76	0.63	0.13
10000	0.4	0.41	-0.01	0.56	0.43	0.13
Relative length of normal seedling root						
0	1	1	0	1	1	0
2000	1	1.08	-0.08	0.93	0.93	0

4000	0.88	0.89	-0.01	0.9	0.85	0.05
6000	1.14	1.23	-0.09	0.91	0.78	0.13
8000	1.16	0.87	0.29	0.85	0.70	0.15
10000	0.14	0.59	-0.45	0.35	0	0.35
Seedling dry weight (g)						
0	0.004	0.003	0.001	0.005	0.007	-0.002
2000	0.003	0.003	0.000	0.006	0.007	-0.001
4000	0.002	0.002	0.000	0.004	0.006	-0.002
6000	0.003	0.003	0.000	0.005	0.005	0.000
8000	0.002	0.001	0.001	0.004	0.005	-0.001
10000	0.001	0.001	0.000	0.002	0.001	0.001
Normal seedling dry weight (g)						
0	0.006	0.007	-0.001	0.004	0.005	-0.001
2000	0.006	0.006	-0.000	0.005	0.005	-0.000
4000	0.005	0.005	0.000	0.003	0.004	-0.001
6000	0.005	0.005	0.000	0.003	0.003	0.000
8000	0.004	0.003	0.001	0.003	0.003	-0.000
10000	0.001	0.001	0.000	0.001	0.000	0.001

Note: Not significantly different based on LSD test at $\alpha = 0.05$ using STAR software.

The largest difference of vigor index and germination percentage based on delta values of tolerant and susceptible genotypes was found at 4000 ppm. The tolerant variety was estimated to have ABA and synthesizing high proline in the seed. ABA and proline according to Ashraf (1994) were able to maintain the cell turgor, water potential in high cells so that the enzyme activity in the germination process was not interfered. Adaptation of tolerant genotype according to Momayezi et al. (2009) in salinity stress was made by accelerating the germination process. Susceptible genotype according to Vibuthi et al. (2015) decreased in the germination and speed of germination. Salinity of 8000 ppm and 10000 ppm NaCl solution showed that the susceptible and tolerant genotypes were the same. It was estimated that the osmoregulation mechanism to maintain the internal osmotic potential was unable to maintain the imbibitions rate so that the seeds germinate slowly and the normal seedling were decreasing. The maximum growth potential of tolerant and susceptible genotypes were not different in all concentrations of NaCl solution. It was in accordance with Mahmud et al. (2016) that the maximum growth potential of tolerant and susceptible genotypes were not different until the concentration of NaCl was 10000 ppm.

Salinity as stated by Kandil et al. (2012) interferes the development of plumule and radicle. The radicle and plumule length (Table 2), and the decrease radicle and plumule length (Table 3), and relative root lengths (Table 4) of tolerant and susceptible genotypes in all concentrations of NaCl solution using rolled paper towel test method showed no significant difference. Those parameters at the concentration of NaCl solution <6000 ppm were decreasing low, but at > 6000 ppm decreased highly. It is estimated due to the toxic effects of salinity, where the accumulation of Na^+, high ratio of Na^+/ K^+ and the decreasing of K^+ and NO_3^- on plumule and radicle (Azarin et al., 2016), would decrease the water potential and cell turgor for the tissue cell expansion of radicle and plumule (Alam et al., 2004). The accumulation of Na^+, Na^+/ K^+ ratio and Cl^- at concentration of 6000 ppm according to Azarin et al. (2016) was low and did not decrease K^+ and NO_3^- for the growth of radicle and plumule, but at salinity higher than 6000 ppm Na^+ and Cl^- it was a cumulated high. The susceptible and tolerant genotypes had similar seedling dry weight in all NaCl concentrations (Table 4). Presumably this was due to the similar radicle and plumule growth of susceptible and tolerant genotypes in all concentrations of NaCl solution. The similarity radicles and plumules growth caused the dry weight of tolerant and susceptible genotypes of plants to be relatively the same. It made the toxic effects of NaCl especially Cl^- ions reported by Islam and Karim (2010); Azarin et al. (2016) would reduce the biomass growth while rice resistance has not occurred.

Top Paper Test

The vigor index, germination percentage, and maximum growth potential of tolerant and susceptible rice genotypes in the top paper test method are presented in Table 5. The tolerant and susceptible genotype vigor index was significantly different at concentrations of 2000 and 4000 ppm of NaCl solution, but relatively similar at 0, 6000, 8000, and 10000 ppm. The difference vigor index of susceptible and tolerant genotype on top paper test method of 2000 and 4000 ppm NaCl solution concentrations were very small at 7.7%. Germination percentage and potential growth of susceptible and tolerant genotypes could not be distinguished in all concentrations of

NaCl solution. The maximum growth potential of tolerant and susceptible genotypes on the rolled paper towel test method also showed no difference.

This showed that it was very difficult to distinguish tolerant and susceptible genotypes based on the maximum growth potential. Radicle and plumule length (Table 2), and the decreas radicle and plumule length (Table 3), the root length relative, and the root dry weight (Table 4) of tolerant and susceptible genotypes in all concentrations of NaCl solution of top paper test method showed no significant difference. The same results indicated that these parameters were unable to distinguish tolerant and susceptible genotypes on the rolled paper towel test method. Salinity of 4000 ppm NaCl in rolled paper towel test was able to distinguish tolerant and susceptible genotypes based on the vigor index and germination percentage. Salinity of 4000 ppm NaCl in top paper test was able to distinguish tolerant and susceptible genotypes based on the vigor index. The concentration of 4000 ppm of NaCl solution in rolled paper towel test showed significantly different delta values between tolerant and susceptible genotypes. The rolled paper towel test method with 4000 ppm NaCl using vigor index and germination percentage were the selected method. The selected method was used to identify the tolerance of 36 rice breeding lines at germination stage and a correlation test using a standard testing on the vegetative stage in the greenhouse were used.

Salinity Tolerance Test on Rice Breeding Lines

Salinity tolerance of rice breeding lines at germination and vegetative stages is presented in Table 5. Vigor index and germination percentage of rice breeding lines in the rolled paper towel test varied widely. The values of vigor index, germination percentage and percentage of dead leaves were based on the average and range spread in the susceptible checks, tolerant checks, and between the range of susceptible checks and tolerant checks. It indicated that the tested breeding lines could be used to test the suitability of the rolled paper towel test method at germination stage through the greenhouse method at vegetative stage. The percentage of dead leaves in the vegetative stage according to Sulaiman (1980) became the main character in determining the salinity tolerance of rice. The tolerance of rice based on the percentage of dead leaves according to Sulaiman (1980) could be classified into four groups of tolerance, i.e. tolerant (0% - 50%), moderately tolerant (51% - 70%), moderately susceptible (71% - 90%), and susceptible (> 90%). The vigor index and germination percentage showed a negative correlation value with the percentage of dead leaves (Table 6). Germination percentage closely correlated with the percentage of dead leaves, -0.72. It indicated that the germination percentage was able to approach the results of the variables of dead leaves rate of the breeding line tested at vegetative stage.

Table 5 Characteristic growth of different rice genotypes under 4000 ppm NaCl at germination and vegetative stages

Genotype[1]	Statistics	Germination		Vegetative
		VI (%)	GP (%)	DL (%)
Breeding lines (36)	Average	20.5	43.4	74.1
	Range	9.3 - 33.3	22.4 - 66.7	17.5 - 97.9
Susceptible Check (2)	Average	12.7	29.3	98.8
	Range	12 - 13.3	27.3 - 31.3	97.5 – 100
Tolerant Check (2)	Average	38.67	67.7	57.1
	Range	32 - 45.3	64 - 71.3	49.2 – 65
Combination (40)	Average	21.0	43.8	74.5
	Range	9.3 - 45.3	22.7 - 71.3	17.5 – 100

Note: VI: vigor index, GP: Germination Percentage, DL: Dead leaves. [1]figure inside () indicates the number of breeding lines.

Table 6 Correlation coefficient of germination test parameters with vegetative stage test parameters

Parameter of vegetative stage test	Parameter of germination stage test	Genotype		
		Breeding line	Check	Combination
Dead leaves(%)	Vigor index (%)	-0.59*	-0.90*	-0.62*
	Germination Percentage (%)	-0.71*	-0.88*	-0.72*
	Radicle length (%)	-0.19	-0.53	-0.23

Note: * Significant at level 0.05

Table 7 Average germination percentage and percentage of dead leaves of the ten most saline tolerant rice breeding lines

No	Breeding lines	GP	Breeding line	DL
1	1121-Ski-1	66.67	BP14082-2b-2-5-TRT-35-1-SKI-4	17.50
2	BP14082-2b-2-5-TRT-35-1-SKI-4	66.67	1121-Ski-1	42.50
3	1131-Ski-4	66.67	1131-Ski-4	46.25
4	BP14082-2b-2-3-TRT-23-4-SKI-2	64.00	BP14080-5b-6-5-TRT-27-2-SKI-2	56.88
5	1127-Ski-4	58.67	BP14082-2b-2-3-TRT-23-4-SKI-2	57.92
6	BP14082-2b-2-5-TRT-35-5-SKI-2	56.00	1127-Ski-4	59.38
7	BP14080-5b-6-5-TRT-27-2-SKI-2	52.00	BP14082-2b-2-5-TRT-35-5-SKI-2	60.00
8	BP14082-2b-2-5-TRT-35-2-SKI-1	52.00	1129-Ski-2	63.75
9	1127-Ski-5	51.33	BP14080-5b-6-5-TRT-26-1-SKI-1	66.25
10	BP14080-5b-6-5-TRT-26-1-SKI-1	50.67	1127-Ski-5	68.13
	Pokalli*	71.33	Pokalli*	49.17

Note: GP: Germination Percentage(%), DL: Dead leaves (%), * tolerant check.

Rice Breeding Lines Tolerant to Salinity

The tolerance test for salinity at vegetative stage could classify the tested breeding lines into 3 groups: tolerant, moderate, and susceptible. The ten most tolerant breeding lines at germination and vegetative stage were selected based on the germination percentage and percentage of dead leaves. The germination percentage and percentage of dead leaves were selected because they were well correlated. The tolerance of rice breeding lines tested based on germination percentage was categorized into 3 tolerant groups that were susceptible (27% - 42%) 17 lines, moderate (42.1% - 57%) 12 lines, and tolerant (57.1% - 71%) 7 lines. The tolerance group was determined by dividing the range of the most susceptible and most tolerant check germination percentage into 3 groups. The tested breeding lines based on the percentage of dead leaves appeared to be 3 tolerant lines, 10 moderately tolerant lines, 15 moderately susceptible lines, and 8 susceptible lines. Eight of the 10 most tolerant breeding lines of germination stage testing were also included in the ten most tolerant breeding lines on vegetative stage testing (Table 7). It indicated that the suitability level of the testing method of germination stage and vegetative stage was 80%. This suitability was higher than the correlation coefficient value of 0.72. It is assumed that there are still several lines showing different salinity tolerance in both stages thus affecting the correlation value.

Conclusion

Rolled paper towel test and top paper testing method on germination test with 4000 ppm NaCl were able to differentiate the tolerant and susceptible rice genotypes based on the vigor index and germination percentage. The results of the salinity tolerance test through rapid test method at germination stage were closely correlated with the results of greenhouse testing method at vegetative stage. The selection of the 10 most tolerant breeding lines of 36 rice breeding lines using the rapid test method at germination stage and the greenhouse method was 80% conformity. The rolled paper towel test germination test treated with 4000 ppm of NaCl solution could be used as a rapid test for rice genotypes salinity tolerance.

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