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Tolerance of 20 Heirloom Rice Varieties at Seedling Stage Salinity Stressed and Their Growth in Lowland Coastal Area

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ABSTRACT

Coastal area of Bengkulu Province is home of many heirloom rice varieties and play an important role in contributing rice production in the province. However, as the climate change proceeds, a progressive sea water inundation has increased soil salinity mainly in low-lying areas and jeopardizing the sustainability of rice production in coastal regions. The use of salt-tolerant varieties, therefore, would be a sensible solution to alleviate the adverse effect of soil salinity in respect of maintaining the crop production in the coastal areas. The objective of this study was to examine the salinity tolerance of 20 heirloom rice varieties collected from a coastal area of Bengkulu Province. Prior to the screening process, lethal concentration 90 (LC90) was determined by assaying the seedlings of 'Kuning Tinggi' in a series of nutrient solution containing a different concentration of NaCl (0, 2000, 4000, 6000, 8000 and 10000 ppm). Using a regression analysis, LC90 was detected at a concentration of 3910 ppm. The screening was performed by exposing the varieties in the nutrient solution culture containing 4000 ppm NaCl and growing them on the tidal swamp. Under nutrient culture evaluation, the symptom of NaCl toxicity was scored and converted to salinity tolerance index. 'Humbur', 'Kuning Tinggi' and 'Padang Bakung' exhibited medium tolerant, while 'Beram', 'Imperata' and 'Kuning' exhibited very sensitive. Further evaluation of the tidal swamp for vegetative and generative performances signified that 'Humbur' and 'Kuning Tinggi' had medium tolerant to salinity stress.

INTRODUCTION

Soil salinization due to tidal inundation along with the rising sea-level has been a serious threat to the rice production in coastal rice growing areas (Mantri et al, 2012; Hoang *et al*., 2015; Shrivastava, et al., 2015). It has been reported that soil salinity at electrical conductivity (EC) of 4 dSm^{-1} could reduce the rice yield up to 15.55% (Dasgupta *et al*., 2014) and the yield loss was increased to 50% as the EC reaches 7.4 dS m^{-1} (Grattan *et al*., 2002).

A saline soil can serve as a reservoir for a number of soluble salts such as Ca^{2+}, Mg^{2+}, Na^{+} and anions SO_4^{2-}, Cl^-, HCO^{3-} with exceptional amounts of K^+, CO_3^{2-}, and NO^{3-} (USDA, 2008). High concentration of those ions, especially Na^+, can cause both osmotic and ionic stresses on rice plant which result in the plant growth reduction and premature leaf senescence (Horie et al., 2012). The rice response to salinity stress varies during the

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plant developmental stages (Wassmann et al, 2009). The seedling and reproductive stages are considered as the most sensitive period to salt stress (Li and Xu, 2007). Leaf drying from the tip downward and from the edge toward the middle were the most noticeable symptoms of salinity stress during the vegetative stage and necrotic flag leaf during the generative stage.

Rice is among a few crops that can grow on salt-affected soil. Rice plants generally tolerate salt by mainly two mechanisms, ion exclusion and osmotic tolerance (Munns and Tester, 2008). These mechanisms can also be further classified into ion exclusion, osmotic tolerance and tissue tolerance (Roy et al, 2014). However, the degree of tolerance to salinity is dictated by the genetic nature of the plant (Mohammadi-Nejad et al., 2012; Hosseini et al., 2012). The use of salinity-tolerant varieties, therefore, would be a sensible solution to alleviate the adverse effect of soil salinity in respect of maintaining the crop production in the coastal areas.

A breeding program addressed to develop new rice varieties for best suited on the salinity prone areas should be devised with salinity tolerant characteristics. There are a large number of genotypes can be used as a source of genes (Herison, et al., 2018) for salinity tolerant, including the heirloom varieties traditionally grown in the Bengkulu coastal areas, that could be incorporated to high yielding varieties. Nonetheless, salinity tolerance is a complex trait (Ahmadizadeh et al., 2018) and no single measure could be used to determine the degree of salinity tolerance in rice. Consequently, effective and efficient screening method should be developed to enable of determining the potential genotypes in a large number array of breeding materials. This study was undertaken to examine the salinity tolerance of 20 heirloom rice varieties collected from a coastal area of Bengkulu Province.

MATERIALS AND METHODS

Genetic materials and experimental sites

Twenty heirloom rice varieties collected from the swampy areas along the coast of Bengkulu Province, Indonesia were evaluated for their tolerance to salinity stress. The experiment was carried out in three phases, namely determination of lethal concentration 90 (LC90), assessment of salinity tolerance, and field evaluation for growth and yield performances. The first two experiments were conducted in the greenhouse of the Agronomy Laboratory, Faculty of Agriculture, Universiy of Bengkulu, while the third experiment was conducted in the tidal swamp area of Kuala coast, Rawa Makmur, City of Bengkulu.

Determination of LC90

The determination of LC 90 was carried out by assaying 'Kuning Tinggi' on Hoagland nutrient culture (Hoque et al., 2015) with different concentrations of NaCl (0, 2000, 4000, 6000, 8000, and 10000 ppm). Fifteen seedlings of one week old were arranged on a punched Styrofoam sheet and floated onto the nutrient solution. The solution pH maintained at 5.8 by adding NaOH 1 N or HCl 1 N at two days interval. The observation was made for the percent of plant mortality after the seedlings were cultured in the nutrient solution for 8 days. A probit analysis was employed to the collected data were evaluate the plant response to the increasing NaCl concentration in the nutrient solution and to determine the LC90.

Assessment of salinity tolerance

The experiment was set up on a nutrient solution with the concentration of NaCl at LC90. For each variety, three sets of ten seedlings of one week old were arranged on a punched Styrofoam sheet and floated onto the nutrient solution. The solution pH was maintained as in the determination of LC90. The plant's height was measured at 4 and 8 days after the seedlings were cultured in the nutrient solution.

The symptom of salinity stress was also observed at 8 days after the seedlings were exposed to the treatment and the degree of severity was scored using modified standard evaluating score (SES) in rating the visual salt injury at seedling stage (Gregorio et al., 1997). The resulting scores were then converted to tolerance indices (TI) as suggested by Tiwari *et al*. (2012).

$$TI = \frac{\sum i(\mathrm{n} \times \mathrm{v})}{\mathrm{N} \times 9} x100\%$$

where TI =tolerance index, n = number of plants in each score, v = the score, N = total number of the observed plants. The salinity tolerance was deemed as highly tolerant (TI = 0%), tolerant (TI = 1 – 10%), modium tolerance (TI = 11– 25%), susceptible (26 – 50%), and highly susceptible (IT ≥ 50%) (IRRI, 1996).

Field evaluation for growth and yield performances on tidal swamp low-land

The experiment was conducted on the tidal swamp rice field with water electric conductivity (EC) = 2-5 dSm^{-1} and increased to 8.37 dSm^{-1} as the seawater inundated the field for three days. A randomized complete block design with three replications was used to allocate the varieties on the 4m x 5m experimental plots with the blocks were 1m spaced apart. One-month-old seedlings from each variety were transplanted to the experimental plot with 25cm x 25cm planting space. Basal fertilizers consisted of 40 kg Urea ha^{-1}, 100 kg SP36 ha^{-1}, and 100 kg ha^{-1} were applied at 7 days after transplanting (DAT). Additional 60 kg Urea ha^{-1} applications were implemented at 21 and 35 DAT.

Scores of salinity tolerance were obtained at tiller formation (vegetative) and heading stage (generative) using the method adopted in the nutrient solution experiment. Samples of ten plants were taken randomly at harvest from each plot for the observation of plant height, leaf number, tiller number, panicle length, number of filled grain/panicle, weight of filled grain/panicle, 100-grain weight, and grain yield/clump. The data from the sampled plants were subjected to analysis of variance and mean separation using the Scott-Knott clustering method. Simple correlation analysis was performed to measure the degree of association between the observed traits.

RESULTS AND DISCUSSION

Effect of different levels salinity on seedling growth

The assay of the seedling response to the different levels of salinity indicated that the stress to salinity was intensified as the salt concentrations were increased. However, no plant mortality was detected until the salt concentration reached 2000 ppm and 100% plant mortality was attained as the salt concentration reached 2000 ppm. The performed probit analysis showed that the LC90 occurred at 3910 ppm. Therefore, NaCl at 4000 ppm was used in preparing the nutrient solution for the following screening experiment.

Assessment of salinity tolerance in the nutrient solution

No variety could be categorized as tolerant or highly tolerant to salinity as the varieties were exposed to 4000 ppm NaCl for 8 days (Table 1). Most of the varieties fell into the category of susceptible or highly susceptible to salinity. Humbur, Kuning Tinggi, and Padang Bakung were the only varieties exhibited medium tolerant to salinity.

The measurement of plant height after 4 and 8 days after the seedlings cultured in the nutrient solution containing 4000 ppm NaCl indicated that the plant growth was persisted at varying degree (Table2). Nevertheless, no particular pattern was found in regard to the association between salinity tolerance and the early seedling growth. This notion was also signified by simple correlation analysis between the two variables where the coefficient of correlation (r) was only 0.24.

The field performances of the plants

As it has been detected in the nutrient solution culture experiment, there was no variety could be deemed to be tolerant or highly tolerant to salinity in the tidal swamp field both during in the vegetative stage and the generative stage (Table 3). Humbur and Kuning Tinggi showed their consistency as moderately salinity tolerant varieties during the seedling, vegetative, and generative stages. On overall, low associations in salinity tolerance were found between seedling stage and vegetative stage (r = 0.24) and between seedling stage and generative stage (r = 0.14). On the other hand, a strong association in salinity tolerance between the vegetative stage and generative stage (r = 0.85). The only exception was found on Cina Abang, Cina Putih, and Pandak Kelabu that showed a shift their salinity tolerance from susceptible during vegetative stage to medium tolerant in the generative stage

Table 1. Scores of salinity stress, tolerance indices, and salinity tolerance among 20 Bengkulu heirloom varieties based on seedlings response to 4000 ppm NaCl

No.	Variety	Score of salinity stress					Tolerance index (%)	Salinity tolerance
		1	3	5	7	9		
1	Bangkok		12	1		2	30.37	susceptible
2	Beram		3	10	2		54.07	highly susceptible
3	Cantik	8	5			2	30.37	susceptible
4	Cina Abang	4	11				27.41	susceptible
5	Cina Putih	5	10				25.93	susceptible
6	Cisadane	5	6	1	1	2	39.26	susceptible
7	Humbur	10	5				18.52	medium tolerant
8	Imperata	3	1	3		8	68.89	highly Susceptible
9	Kuning	1	4	7		3	55.56	highly Susceptible
10	Kuning Air Dingin	4	8	1	2		34.81	susceptible
11	Kuning Pendek	5	5	4	1		34.81	susceptible
12	Kuning Pendek 2	6	3	3	1	2	40.74	susceptible
13	Kuning Sualowangi	1	14				31.85	susceptible
14	Kuning Tinggi	10	5				18.52	medium tolerant
15	Padang	4	10	1			28.89	susceptible
16	Padang Bakung	10	5				18.52	medium tolerant
17	Pandak Kelabu		10	5			40.74	susceptible
18	Pandan Wangi	5	10				25.93	susceptible
19	Pondok Batu	2	4	6	2	1	49.63	susceptible
20	Simpat Abang	5	9	1			27.41	susceptible

Table 2. Mean values of the plant height of 20 Bengkulu heirloom varieties at 4 and 8 days after their seedlings cultured in the nutrient solution containing 4000 ppm Na Cl

No.	Variety	Plant height (cm)				Plant height increment (cm)
		4 days		8 days		
1	Bangkok	9.07	± 0.31	14.49	± 2.29	5.42
2	Beram	14.73	± 0.66	17.03	± 4.15	2.30
3	Cantik	11.24	± 1.34	16.95	± 1.63	5.71
4	Cina Abang	14.31	± 2.07	16.05	± 0.77	1.74
5	Cina Putih	16.41	± 1.88	19.60	± 0.78	3.19
6	Cisadane	10.81	± 1.28	17.41	± 1.72	6.60
7	Humbur	9.74	± 0.75	15.67	± 0.23	5.93
8	Imperata	5.79	± 0.52	12.43	± 1.22	6.64
9	Kuning	9.41	± 0.92	15.41	± 1.05	6.00
10	Kuning Air Dingin	13.49	± 0.16	15.53	± 1.21	2.04
11	Kuning Pendek 1	7.21	± 0.70	13.63	± 0.38	6.42
12	Kuning Pendek 2	8.78	± 0.52	16.44	± 0.68	7.66
13	Kuning Sulaowangi	15.75	± 1.72	18.08	± 0.64	2.33
14	Kuning Tinggi	13.13	± 0.46	16.1	± 1.43	2.97
15	Padang	14.71	± 1.49	18.75	± 1.61	4.04
16	Padang Bakung	11.97	± 1.08	15.34	± 1.25	3.37
17	Pandak Kelabu	15.96	± 0.45	21.71	± 0.13	5.75
18	Pandan Wangi	6.73	± 0.30	14.29	± 0.54	7.56
19	Pondok Batu	8.56	± 0.89	14.35	± 1.16	5.79
20	Simpat Abang	7.15	± 0.88	14.19	± 1.15	7.04

Analysis of variance indicated significant variations among the varieties for all observed plant traits. Table 4 displays the mean of growth performances among the varieties as grown on tidal swamp field and grouped by Scott-Knott clustering. There were four groups of varieties on the basis of plant height. Group A was comprised of 4 semi-dwarf varieties; Group B was comprised of 8 intermediate by intermediate varieties; Group C was comprised of 5 tall varieties); Group D was comprised of 3 very tall varieties. Four groups of tillering ability were also revealed among the varieties. Group A and Group B could be categorized as

Table 3. Scores of salinity stress, tolerance indices, and salinity tolerance among 20 Bengkulu heirloom varieties as grown on a tidal swamp field

No.	Variety	Vegetative stage		Generative stage	
		Tolerance index (%)	Salinity tolerance	Tolerance index (%)	Salinity tolerance
1	Bangkok	65.93	highly susceptible	65.19	highly susceptible
2	Beram	85.19	highly susceptible	80.74	highly susceptible
3	Cantik	80.74	highly susceptible	85.19	highly susceptible
4	Cina Abang	31.85	susceptible	21.48	medium tolerant
5	Cina Putih	36.30	susceptible	23.70	medium tolerant
6	Cisadane	41.48	susceptible	41.48	susceptible
7	Humbur	24.44	medium tolerant	22.96	medium tolerant
8	Imperata	48.15	susceptible	45.93	susceptible
9	Kuning	48.15	susceptible	44.44	susceptible
10	Kuning Air Dingin	43.70	susceptible	33.33	susceptible
11	Kuning Pendek	39.26	susceptible	27.41	susceptible
12	Kuning Pendek 2	51.11	susceptible	25.19	susceptible
13	Kuning Sulaowangi	42.22	susceptible	40.00	susceptible
14	Kuning Tinggi	22.96	medium tolerant	17.04	medium tolerant
15	Padang 2	76.30	highly susceptible	67.41	highly susceptible
16	Padang Bakung	46.67	susceptible	34.81	susceptible
17	Pandak Kelabu	28.89	susceptible	20.00	medium tolerant
18	Pandan Wangi	51.11	highly susceptible	89.63	highly susceptible
19	Pondok Batu	42.22	susceptible	32.59	susceptible
20	Simpat Abang	57.04	highly susceptible	48.89	susceptible

having medium tillering ability and each comprised of 8 varieties. Group C dan Group D were considered as good tillering ability and each comprised of 2 varieties. For leaf number, five groups of varieties were detected and it appeared that leaf number was related to the tiller number born in the clump. The more tiller number born in the clump, the higher number leaf would be formed. The performed correlation analysis showed that leaf number was strongly associated with tiller number (r = 0.85), but weakly associated to plant height (r = -0.35). Similarly, tiller number was weakly associated to plant height (r = -0.33).

Table 4. Mean values of the vegetative characteristics exhibited by 20 Bengkulu heirloom varieties as grown on a tidal swamp field

No.	Variety	Plant height (cm)	Tiller number	Leaf number
1	Bangkok	118.68 b	23.76 d	140.6 e
2	Beram	186.24 d	16.40 a	88.6 a
3	Cantik	165.42 d	16.96 a	98.4 a
4	Cina abang	136.59 c	18.40 b	115.5 c
5	Cina putih	138.71 c	18.13 b	112.8 c
6	Cisadane	111.07 a	23.24 d	141.0 e
7	Humbur	160.87 d	16.93 a	96.9 a
8	Imperata	121.65 b	18.26 b	107.0 b
9	Kuning	121.01 b	19.74 c	114.5 c
10	Kuning Air Dingin	121.43 b	17.80 b	106.0 b
11	Kuning Pendek	116.89 b	16.80 a	100.8 b
12	Kuning Pendek 2	106.49 a	17.07 a	106.2 b
13	Kuning Sulaowangi	131.87 b	15.10 a	83.7 a
14	Kuning Tinggi	122.23 b	20.60 c	125.1 d
15	Padang 2	111.14 a	15.40 a	86.2 a
16	Padang Bakung	133.85 b	16.68 a	99.1 a
17	Pandak Kelabu	136.99 c	18.47 b	116.2 c
18	Pandan Wangi	111.01 a	18.08 b	96.3 a
19	Pondok Batu	140.77 c	18.19 b	106.2 b
20	Simpat Abang	135.31 c	17.54 b	102.1 b

Mean values in a column followed by the same letter are not significantly different by Scott-Knott cluster analysis at 5% level.

Table 5. Mean values of the yield characteristics exhibited by 20 Bengkulu heirloom varieties as grown on a tidal swamp field

No.	Variety	Panicle length (cm)	Filled grain number panicle^{-1}	Filled grain weight panicle^{-1} (g)	100 grain weight (g)	Grain yield clump^{-1} (g)
1	Bangkok	24.14 a	382.16 b	4.76 b	2.45 c	50.33 f
2	Beram	29.95 c	510.83 b	2.33 a	1.65 b	21.62 b
3	Cantik	27.44 b	125.16 a	2.56 a	0,47 a	6.71 a
4	Cina Abang	24.75 a	427.60 b	4.26 b	3.10 d	49.36 f
5	Cina Putih	24.57 a	455.80 b	4.70 b	2.49 c	36.07 d
6	Humbur	23.90 a	334.33 b	4.58 b	2.09 c	28.32 c
7	Imperata	26.46 b	163.16 a	2.53 a	0.65 a	9.72 a
8	Kuning	24.70 a	493.54 b	4.60 b	2.84 d	48.08 f
9	Kuning Air Dingin	27.08 b	405.61 b	4.07 b	2.43 c	36.87 d
10	Kuning Pendek	26.27 b	956.00 c	3.16 a	1.23 b	16.70 b
11	Kuning Pendek 2	26.71 b	521.87 b	2.20 a	0.61 a	7.96 a
12	Kuning Sulaowangi	22.85 a	132.90 a	2.31 a	1.52 b	12.31 a
13	Kuning Tinggi	24.71 a	282.67 a	4.60 b	2.22 c	39.85 e
14	Padang 2	23.47 a	218.62 a	6.79 c	1.29 b	14.97 b
15	Padang Bakung	24.94 a	404.82 b	4.56 b	2.53 c	32.74 d
16	Pandak Kelabu	24.97 a	370.47 b	3.90 b	2.50 c	40.56 e
17	Pandan Wangi	26.39 b	296.98 a	2.10 a	0.97 b	10.68 a
18	Pondok Batu	32.20 d	160.57 a	2.26 a	0.35 a	5.47 a
19	Putih Cisadane	25.43 a	344.74 b	4.58 b	1.46 b	29.05 c
20	Simpat Abang	24.30 a	269.44 a	5.07 b	1.55 b	22.85 b

Mean values in a column followed by the same letter are not significantly different by Scott-Knott cluster analysis at 5% level.

Table 5 presents the performances of yield and yield attributed traits among the varieties as grouped by Scott-Knott clustering. As viewed from the grain yield clump^{-1}, there were 6 groups were detected among the varieties. The group with higher grain yield (Group F) consisted of Bangkok, Cina Abang, and Kuning. These varieties were mostly characterized by higher filled grain weight panicle^{-1} and 100-grain weight as suggested by correlation analysis, where grain yield clump^{-1} was strongly associated to filled grain weight panicle^{-1} and 100-grain weight with r = 57 and r = 94, respectively.

Rice is a salinity sensitive crop and most of varieties loss their yield as the salinity reaches a threshold EC level at 3 dSm^{-1}. Up to 50% yield loss could be expected if the salinity is raised to EC level at 7.2 dSm^{-1} (Hoang et al., 2016). Juvenile stage (Lutts et al, 1995) was found as the most sensitive stage to high salinity. In this study, the symptoms of salinity stress starting to appear at 4 days after the seedling cultured in the nutrient solution containing 1000 ppm NaCl. At this concentration, the symptom was leaf tips rolling and followed by the plant death as the stress was prolonged or the concentration was increased. Leaf rolling is presumed as avoiding mechanism in respect to the toxic effect of NaCl by reducing the plant transpiration (Platten et al., 2013; Kumar et al., 2013; Reddy et al, 2017) and retarding the Na^+ transport to the leaf tissues (Yeo at al., 1990). The performed probit analysis on 8 days after the seedling cultured in nutrient solution indicated that LC90 was attained as the nutrient solution contained 3910 ppm of NaCl. Consequently, 4000 ppm was used as the NaCl concentration for salinity tolerance screening at the seedling stage (Utama, 2010).

The screening on the seedlings of 20 Bengkulu heirloom varieties for salinity tolerance in nutrient solution indicated that only 3 varieties (Humbur, Kuning Tinggi, and Padang Bakung) showed medium tolerance to salinity. However, only Humbur and Kuning Tinggi showed their consistency as having medium tolerant to salinity during vegetative and generative stages when they were grown on tidal swamp field having EC = 8.37 dS m^{-1} for a week, whereas Padang Bakung showed it susceptibility to salinity in the field. Such inconsistency was also reported (Dika *et al*., 2013). Under field condition, Humbur and Kuning Tinggi showed the mildest symptoms of salinity stress as indicated only by leaf rolling at vegetative stage and flag leaf yellowing at the generative stage. In term of the growth and yield performances, Humbur and Kuning Tinggi were not noteworthy, but they could serve as source of genes for salinity tolerance.

CONCLUSION

In conclusion, the NaCl lethal concentration to 90% of the rice population at seedling stage was detected at 3910 ppm. Screening for salinity tolerance in rice could be conducted in a nutrient solution containing 4000 ppm (EC = 6.25 dS m^{-1}) to permit evaluation of a large number of genotypes. It has been confirmed that results of screening were consistent with the growth and yield performances in the tidal swamp field. Humbur and Kuning Tinggi exhibited as heirloom varieties having medium tolerant to salinity stress and could be used as a source of genes for salinity tolerance for developing high-yielding rice varieties suitable to salinization.

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