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Salinity stress tolerance of advanced swamp rice breeding lines

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Abstract Problem of salinity stress often occurs on rice grown on tidal swamps, especially those located in estuaries near river mouths. Salinity stress can drastically reduce plant growth and yield. The use of varieties better adapted to saline swamplands would lessen the risk of yield reduction or crop failure. The salinity tolerance of 10 advanced swamp rice breeding lines during early plant vegetative growth was reported their agronomic performances on a saline swampland. A greenhouse experiment was performed to grow the lines on a nutrient solution containing different concentrations of NaCl. Similarly, all these genotypes were subjected to field experiments on inland swampland and estuarine swampland. The mortality data on the greenhouse experiment during the first 7 days after transplanting (DAP) showed that the median lethal concentration of NaCl (LC50) ranged from 1963.28 ppm to 4863.44 ppm has placed UBPR 1, UBPR 3, UBPR 4, UBPR 6, and UBPR 8 as tolerant genotypes, UBPR 2, UBPR 7, UBPR 9, and UBPR 10 as medium tolerant genotypes, and UBPR 11 as susceptible genotype. However, as the plants reached 14 DAP the range of LC50 was reduced to between 1456.72 ppm and 3374.26 ppm, and accordingly no breeding lines could be deemed as tolerant genotype The water salinity of estuarine swampland reached 5865.23 ppm had resulted in a substantial reduction in the agronomic performances of all breeding lines. The grain yield of the breeding lines obtained from estuarine swampland was reduced between 25.8% and 52.9% when compared to those obtained from inland swampland, with the highest reduction found on UBPR 8. The current study provides new insight into the development of new rice varieties tolerant to salinity stress.

Keywords: Growth performance, Estuarine swampland, Grain yield, Inland swampland, Median lethal concentration (LC50)

Introduction

Rice is the staple food for most Indonesians and is the main source of calories for activity (75%) (Mardianto, 2017). In 2019, rice consumption in Indonesia reached 25.34 MMT of milled rice (Astuti, 2020) and will increase in

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the coming years as the population grows. It is estimated that by 2035, the total population of Indonesia will reach an estimated 321 million and the annual consumption of milled rice is close to 40 MMT (BPPT, 2016).

Although rice self-sufficiency has been attained in recent years, it will be burdensome to maintain due to the conversion of productive rice fields to other uses continuing to occur and it is not proportionate to the establishment of new rice fields. Despite this situation, Indonesia has a vast swampland area covering 33.4 million hectares, consisting of 20.13 million hectares of tidal swamps and 13.28 million hectares of inland swamps, of which 9 million to 10 million hectares have the potential for crop production (Ritung *et al.*, 2014). If these swamplands are optimized for rice production, they will become an alternative source of food for the Indonesian population.

In the case of the tidal swampland, especially those located in estuaries bordering the coast, the presence of high seawater intrusion has resulted in saline land conditions. If seawater intrusion enters the paddy field, it will cause salinity stress on the plant and result in reducing the productivity and quality of the yield (Amirjani, 2011; Minh *et al*., 2016). Nevertheless, it has been reported that the level of sensitivity to salinity stress varies among the rice genotypes (Sakina *et al.*, 2016; Senguttuvel *et al.*, 2016). It means that the degree of salinity tolerance in rice to a greater extent is determined by the genetic background of the plant. Hence, the development of rice varieties better adapted to salinity-prone environments can be regarded as a worthwhile endeavor to minimize yield loss resulting from salinity stress. The study was conducted to examine the salinity tolerance of 10 advanced swamp rice breeding lines during early plant vegetative growth and to evaluate their agronomic performances on saline swampland.

Materials and methods

Planting materials

Seeds of ten advanced rice breeding lines developed for swampland agroecosystem consisted of UBPR 1, UBPR 2, UBPR 3, UBPR 4, UBPR 6, UBPR 7, UBPR 8, UBPR 9, UBPR 10, and UBPR 11, were used in the present study.

Experimental setup

All the genetic materials were evaluated for their rate of mortality on salinity stress during the early vegetative stage and their relative agronomic performances in saline and non-saline environments. The mortality rate on salinity stress was performed in a greenhouse to grow 14-days-old seedlings of each breeding line on perforated styrofoam in 30 cm x 40 cm plastic basins filled with nutrient solution containing different NaCl concentrations (0, 3000, 6000, and 9000 ppm) as the growing media for 14 days. A split plot design with three replications was employed to allocate the media as the main plot and 10 seedlings of each breeding line as the subplot. Besides that, two field experiments were carried out on two agroecosystems, i.e., estuarine swampland (saline) and inland swampland (non-saline). In each agroecosystem, the breeding lines were allotted on the experimental plots using a randomized complete block design with three replications. The plot sizes were 3 m x 3 m, each containing 144 plants spaced 25 cm x 25 cm. Crop management adopted the common cultivation method of rice.

Data collection and analysis

For the greenhouse experiment, the mortality rate was observed at 7 and 14 days after the seedlings were transplanted to the growing media (DAP). The collected data were used to determine the median lethal concentration of NaCl (LC50) for each breeding line. For the field experiments, five samples of the representative clump in each plot were recorded for plant height, total tiller number/clump, productive tiller number/clump, panicle length, grain number/panicle, percentage of filled grain/panicle, 100-grain weight, and grain yield/clump. In addition, the heading date, harvest date, and grain yield/plot were recorded on a plot basis. The analysis variance for all observed traits was carried out for each agroecosystem, while Scott-Knott cluster analysis was used to group the breeding lines according to their trait performance.

Results

Greenhouse experiment

All seedlings on the growing media containing no NaCl stayed alive and continued to grow until the end of 14 days of observation. However, plant mortality was observed at varying rates as NaCl was added to the media. Visually, the symptoms of salinity stress began with the drying of the tips of the old leaves and then extended to the base of the leaves. Such drying continued from the younger leaves to the whole leaf and was followed by the death of the plant. Rate of plant mortality of the breeding lines as their seedlings were imposed to 3000 ppm NaCl is shown in Figure 1. No plant mortality was found at 1 DAP but it started to occur at 2 DAP as initiated by UBPR 7 and UBPR 11, followed by other breeding lines in the following days. Of the 12 breeding lines evaluated, UBPR 11 was the fastest line to die and all plants died at 10 DAP, while some plants of other lines remained to survive until the last day of observation (14 DAP). UBPR 10 was the line with the lowest plant mortality rate, followed by UBPR 1.

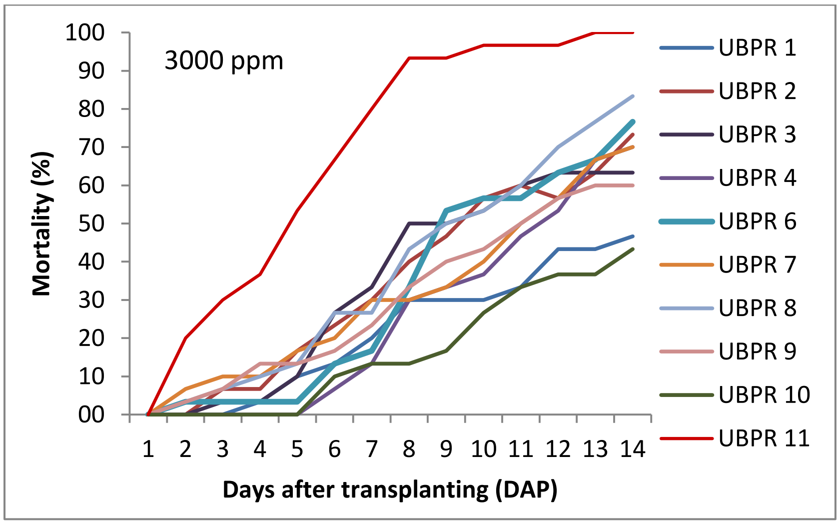

Figure 1. Plant mortality rate in breeding lines on growing media containing 3000 ppm NaCl

As the concentration of NaCl in the growth medium was increased to 6000 ppm, all breeding lines experienced plant mortality at 1 DAP, except for UBPR4, which started to show plant mortality at 2 DAP (Figure 2). As at the concentration of 3000 ppm, plant mortality continued to increase as the days progressed and only six lines (UBPR 1, UBPR 2, UBPR 3, UBPR 4, UBPR 7, and UBPR 8) still left live plants up to 14 DAP. A more extreme appearance was observed in a growth medium containing 9000 ppm, where plant death occurred at 1 DAP in all breeding lines (Figure 3). 100% plant mortality started to occur at UBPR 10 at 6 DAP and was progressively followed by the rest of the breeding lines on the following days until 10 DAP.

The estimates of the median lethal concentration of NaCl (LC50) as presented in Table 1 indicated the salinity tolerance among the breeding lines. The degree of tolerance was set as follows: LC50 > 4000 ppm tolerant, LC50 range from 3000 ppm to 4000 medium tolerant, LC50 range from 2000 ppm to 3000 ppm medium susceptible, and LC50 < 2000 ppm susceptible. Using these criteria, the LC50 at 7 DAP ranged from 1963.28 ppm to 4863.44 ppm has placed UBPR 1, UBPR 3, UBPR 4, UBPR 6, and UBPR 8 as tolerant genotypes, UBPR 2, UBPR 7, UBPR 9, and UBPR 10 as medium tolerant genotypes, and UBPR 11 as susceptible genotype. However, as the plants reached 14 DAP the range of LC50 was reduced to between 1456.72 ppm and 3374.26 ppm, indicating that the tolerance of breeding lines was reduced as the salinity stress was prolonged, and accordingly, no breeding lines could be deemed as tolerant genotype.

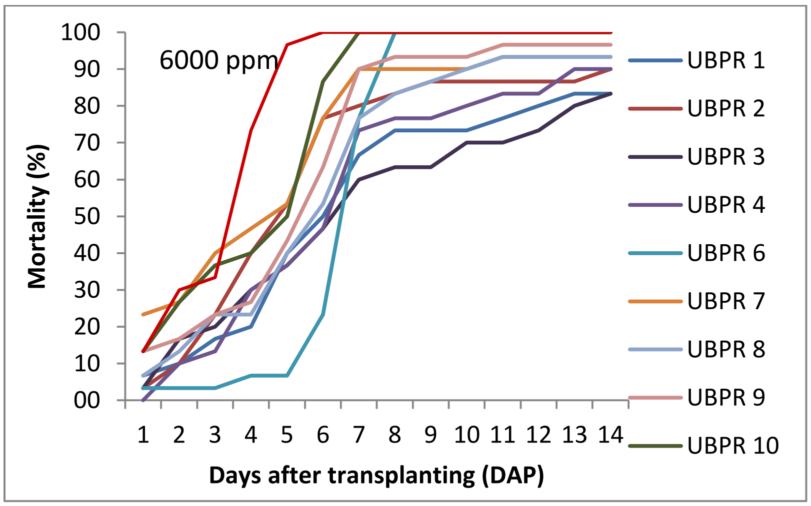

Figure 2. Plant mortality rate in breeding lines on growing media containing 6000 ppm NaCl

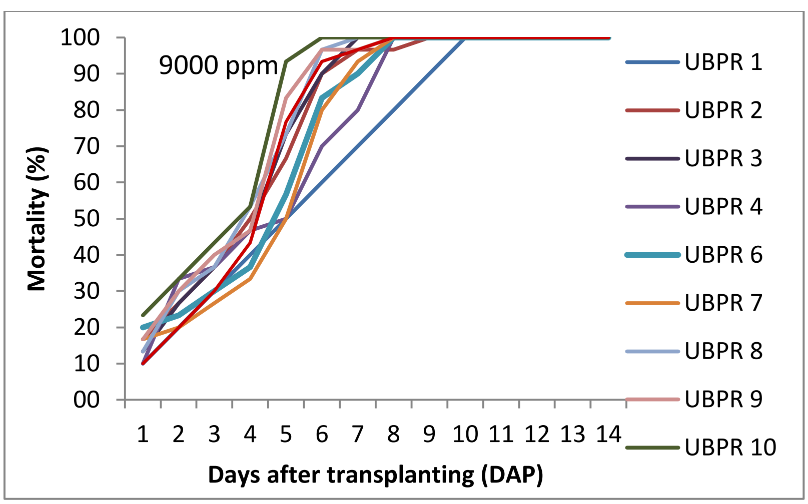

Figure 3. Plant mortality rate in breeding lines on growing media containing 9000 ppm NaCl

Table 1. The estimates of the median lethal concentration of NaCl (LC50) for 10 advanced rice breeding lines at 7 DAP and 14 DAP

No.	Line	LC50 (ppm)	
		7 DAP	14 DAP
1	UBPR 1	4527.46	3204.98
2	UBPR 2	3731.20	2369.12
3	UBPR 3	4849.96	2764.77
4	UBPR 4	4863.44	2451.78
5	UBPR 6	4541.34	2013.23
6	UBPR 7	3458.59	2658.14
7	UBPR 8	4074.16	2063.90
8	UBPR 9	3608.10	3374.26
9	UBPR 10	3935.92	2842.90
10	UBPR 11	1963.21	1456.72

Field experiments

Drought from the tillering stage and occasional ingress of seawater led was increased in NaCl concentration in soil water to 5865.23 ppm. Although irrigation of fresh water pumped from wells had been carried out to meet crop water demands and to reduce the negative effects of salinity, damage to plant growth and decreased seed yield persisted. Table 2 shows the comparative performances of plant growth and development among the breeding lines in both inland swampland and estuarine swampland. In all cases, substantial differences between the two agroecosystems were observed in plant height, total tiller number/clump, heading date, and harvest date. Estuarine swampland produced 28.3% to 58% shorter plants with 26% to 67% less tiller, 8 days to 40 days heading delay, and 6 days to 39 days harvest delay than inland swampland. Among the breeding lines, UBPR 1 had the highest reduction in plant stature, followed by UBPR 3, UBPR 10, and UBPR 4, respectively. UBPR 1 and UBPR 4 had also the highest reduction in total tiller number/clump. UBPR 8 had the longest delay in reaching the heading and harvest stages, followed by UBPR 4.

The yield-contributing traits also exhibited similar performances. All breeding lines grown in estuarine swampland decreased their productive tiller number/clump by 21.7% to 75.6%, panicle length by 7.1% to 36.6%, grain number/panicle by 0.4% to 30.0%, percentage of filled grain/panicle by 44.2% to 69.1%, and 100-grain weight by 1.3% to 30.0% when compared to those in inland swampland (Table 3). As for the number of tillers/clump, the highest reduction in the number of productive tillers/clump occurred in UBPR 1. A large reduction in the number of productive tiller/clump also occurred in UBPR 3, UBPR 4, UBPR 10, and UBPR 11. For panicle length, the highest reduction was found on UBPR2, whereas the highest reduction in grain number/panicle was found on UBPR 3. All breeding lines, except UBPR 11, showed a reduction of more than 50% in the percentage of filled grain/panicle, with UBPR 1 being the highest. Likewise, the highest reduction in 100-grain weight was also observed on UBPR 3.

The grain yields observed from the sampled clumps and plots are listed in Table 4. It can be noted that all breeding lines grown in estuarine swampland experienced a decrease in yield compared to those grown in inland swampland. The average yield loss recorded on sampled clumps ranged from 14.5% to 42%, with the highest being shown by UPBR 4 and followed by UPBR 9, UPBR 8, UPBR 6, and UPBR 2. In addition, yield losses on a plot basis ranged from 25.8% to 52.9%, with UBPR 8 showing the largest decline, followed by UPBR 3, UPBR 2, UPBR 1, and UPBR 7. The change in the rank of the breeding line for the yield loss in both measurement methods was mainly due to the difference in the number of harvestable clumps in the plot. Some plots in estuarine swampland no longer had a population of 100 clumps, as in inland swampland, due to plant mortality during the growing stages. Considering the amount of grain that can be harvested from estuarine swampland, UBPR 10, UBPR 7, and UPBR 6 are more suitable for salinity-affected agroecosystems because their grain yields are higher than other systems.

Discussion

Salinity stress is a major challenge for rice production in agroecosystems prone to seawater ingress. The results of the greenhouse experiment presented in the current study indicated that salinity stress imposed on the advanced rice breeding lines during the early plant establishment resulted in plant mortality. Although variation existed between the breeding lines, the rate of plant mortality increased along with the duration of stress and it was even faster as the NaCl concentration in the growing media got higher. No breeding line survived for more than 10 days when exposed to 9000 ppm NaCl. Similar findings were also reported by Islam and Karim (2010) and Arzie *et al.* (2015). The use of median lethal concentration of NaCl (LC50) at 7 DAP, instead of 14 DAP, should be sufficient to discern the salinity stress tolerance between the breeding lines as also adopted by Prusty *et al.* (2018) and Chakraborty *et al.* (2020).

Table 2. Mean performances of plant growth and development of 10 advanced rice breeding lines grown in inland swampland (IS) and estuarine swampland (ES)

No.	Lines	Plant height (cm)					Total tiller number/ clump					Heading date (DAP)					Harvest date (DAP)				
		IS		ES		Diff. (%)	IS		ES		Diff. (%)	IS		ES		Diff. (day)	IS		ES		Diff. (day)
1	UBPR 1	126.2	a	52.9	d	58.1	35.6	a	11.7	b	67.0	102.0	b	121.0	b	19.0	141.0	a	153	c	12.0
2	UBPR 2	91.1	b	65.3	b	28.3	20.9	b	13.1	b	37.5	87.0	c	106.0	d	19.0	115.7	d	137	e	21.3
3	UBPR 3	139.4	a	59.2	c	57.5	20.0	b	12.9	b	35.4	103.0	b	111.7	c	8.7	138.0	b	145	d	7.0
4	UBPR 4	116.6	a	58.4	c	49.9	31.0	a	12.3	b	60.9	101.0	b	125.7	a	24.7	134.0	c	159	a	25.0
5	UBPR 6	98.5	b	63.4	b	35.6	20.7	b	15.4	a	25.5	79.7	d	95.7	e	16.0	110.0	d	125	g	15.0
6	UBPR 7	98.9	b	63.7	b	35.6	19.0	b	13.2	b	30.6	91.0	c	107.7	c	16.7	120.0	c	137	e	17.0
7	UBPR 8	87.8	b	62.9	b	28.3	21.0	b	14.7	a	30.1	85.0	d	125.0	a	40.0	114.5	d	153	c	38.5
8	UBPR 9	82.8	b	54.9	d	33.7	21.7	b	13.8	b	36.4	83.7	d	94.3	e	10.7	114.7	d	125	g	10.3
9	UBPR 10	125.4	a	62.3	b	50.3	19.6	b	14.5	a	26.1	113.3	a	122.0	b	8.7	144.7	a	154	b	9.3
10	UBPR 11	98.3	b	69.3	a	29.5	23.7	b	15.5	a	34.7	87.7	c	95.7	e	8.0	122.7	c	129	f	6.3

Table 3. Mean performances of yield-contributing traits of 10 advanced rice breeding lines grown in inland swampland (IS) and estuarine swampland (ES)

No.	Lines	Productive tiller number/clump			Panicle length (cm)			Grain number/ panicle			% filled grain/ panicle			100-grain weight (g)		
		IS	ES	Diff. (%)	IS	ES	Diff. (%)	IS	ES	Diff. (%)	IS	ES	Diff.	IS	ES	Diff. (%)
1	UBPR 1	32.8 a	8.0 a	75.6	19.6 b	16.8 c	14.3	114.0 c	88.3 e	22.5	82.9 a	25.6 f	69.1	3.1 a	3.0 a	1.3
2	UBPR 2	14.8 c	10.2 a	31.2	20.8 b	13.2 d	36.6	114.7 c	97.1 d	15.3	79.0 b	28.3 e	64.1	2.4 c	2.1 c	11.4
3	UBPR 3	18.1 c	9.3 a	48.8	22.4 a	19.1 b	14.8	137.0 a	95.9 d	30.0	86.3 a	28.8 e	66.7	2.6 b	1.8 c	30.0
4	UBPR 4	27.6 b	9.0 a	67.3	21.4 b	19.9 b	7.1	106.5 c	100.3 c	5.8	86.6 a	30.9 d	64.3	2.7 b	2.2 c	18.9
5	UBPR 6	14.4 c	11.3 a	21.7	22.9 a	18.6 b	19.0	97.3 d	96.9 d	0.4	79.7 b	34.3 c	57.0	2.1 c	1.7 c	16.9
6	UBPR 7	13.2 c	8.9 a	32.2	24.1 a	22.4 a	7.3	124.7 b	89.7 e	28.1	80.7 b	26.7 f	66.9	2.3 c	2.1 c	7.8
7	UBPR 8	16.6 c	10.5 a	36.9	24.2 a	18.7 b	22.9	116.0 c	92.1 e	20.6	75.5 b	31.2 d	58.7	2.5 c	2.3 b	8.0
8	UBPR 9	15.0 c	9.5 a	36.3	23.5 a	21.8 a	7.3	98.0 d	91.3 e	6.8	86.3 a	36.7 b	57.5	2.5 c	1.8 c	26.7
9	UBPR 10	17.0 c	9.5 a	44.3	23.6 a	19.7 b	16.4	141.7 a	111.3 b	21.4	84.6 a	37.4 b	55.8	2.3 c	1.9 c	16.1
10	UBPR 11	21.4 c	10.3 a	51.8	21.3 b	19.7 b	7.4	138.3 a	126.9 a	8.3	87.2 a	48.7 a	44.2	2.2 c	2.0 c	9.0

Table 4. Mean grain yield/clump and grain yield/plot of 10 advanced rice breeding lines grown in inland swampland (IS) and estuarine swampland (ES)

No.	**Lines**	**Grain weight/clump (g)**					**Grain weight/plot (g)**				
		IS		ES		Diff. (%)	IS		ES		Diff. (%)
1	UBPR 1	44.6	a	34.7	a	22.1	1275.5	a	735.2	b	42.4
2	UBPR 2	33.7	a	23.2	d	31.0	1283.8	a	725.8	b	43.5
3	UBPR 3	34.4	a	24.6	d	28.4	1486.3	a	772.5	b	48.0
4	UBPR 4	28.8	b	16.4	e	42.9	1222.3	b	862.3	a	29.4
5	UBPR 6	19.6	b	12.3	e	37.0	1110.0	b	722.8	b	34.9
6	UBPR 7	40.6	a	32.2	b	20.6	1461.0	a	863.3	a	40.9
7	UBPR 8	39.8	a	24.7	e	38.0	1419.5	a	668.0	b	52.9
8	UBPR 9	29.2	b	17.9	e	38.7	1104.3	b	757.4	b	31.4
9	UBPR 10	36.8	a	27.8	c	24.6	1352.5	a	913.7	a	32.4
10	UBPR 11	20.5	b	17.5	e	14.5	1016.7	b	754.6	b	25.8

Field experiments conducted in inland swampland and estuarine swampland provided comparable growth and yield performances of the examined breeding lines. Consistently, the growth and development of breeding lines have been inhibited due to salinity stress in estuarine swamps, resulting in a sharp decline in grain yield. Since rice is classified as very sensitive to salt stress (Rahman *et al*., 2017; Riaz *et al.*, 2019), the choice of genotype is a critical decision for growing rice in estuarine swamplands. Unless otherwise, complete salinity tolerance varieties are available, those comparatively high grain yields should be taken into consideration to minimize the risk of total crop failure. Moreover, all these findings provide a new insight into further development of new high-yielding rice varieties better adapted to salinity-prone agroecosystems.

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