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Susceptibility of Malaysian Rice (*Oryza sativa* L.) Cultivar to Saline Water Submergence Based on the Morphological Traits

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ABSTRACT

Saline water submergence is a newly emerge abiotic stress jeopardizing rice production especially for the rice fields located nearby or alongside coastal areas. The stress was caused by the intrusion of sea water into those rice fields causing flash flood mainly during monsoon season. The present study was conducted to evaluate susceptibility level of selected Malaysian rice cultivars to saline water submergence at seedling stage based on the morphological traits and survival rate. There were six genotypes involved in the study mainly IR64-*Sub1* as submergence tolerant control, Pokkali as salinity tolerant control, IR64 as susceptible control and MR297, MR284 and MR253 as local rice cultivars, respectively. The experiment was conducted using split plot design with three replications. On the day 14 after germination, all rice seedlings were totally submerged of about one-meter depth in a polyethylene tank containing saline water at 0, 4, 8 and 12 dS/m for 14 days while the non-submerged plant was control of the experiment. Seedling growth attributes and survival rate were recorded before, right after de-submerged and 14 days after de-submerged. All genotypes however were susceptible to saline water submergence at 4, 8 and 12 dS/m. In contrast, under 0 dS/m, IR64-*Sub1* recorded significantly higher survival rate at 83% as compared to MR284 (17%), MR297 (17%), Pokkali (8%), MR253 (0%) and IR64 (0%). All genotypes were not survived under saline submergence. Therefore, further phenotypic screening of rice genetic resources originated from or nearby coastal areas could be suggested in order to increase chance of identifying potentially tolerant genotype to saline water submergence.

Keywords: Rice, saline water submergence, tolerant genotype, morphology

INTRODUCTION

Rice is one of the main sources of carbohydrate for about 3 billion of world population especially people living in the Asian region and narrow down in Malaysia, rice is regarded as staple food for local (Nguyen, 2002). Rice also had been reported to contribute 50 to 80% of daily calories (Zhang et al., 2010). Climate uncertainties and weather changing pattern has crucial impact to agricultural sector. Due to poorly constructed development along the coastline, the problem like coastal erosion arose because of sea level increment which led to high tide. Hence, it is estimated that by the year 2050, saline water is going to penetrate further inland and change the geography in the deltas and coastal plains on an alarming scale (The Food and Agriculture Organization of the United Nations, 2011). The seawater may overflow and easily get into low-lying area which led to flooding of the residential and agricultural areas. The seawater intrusion into those areas especially during monsoon season was concurrently mixed together with the normal fresh water hence could be regarded as brackish water. Based on our field sampling at the rice fields located nearby coastal area in Pekan, Pahang, Malaysia during monsoon floods in the year 2018 and 2020, the level of salinity of the intruded brackish water was recorded in between 2 dS/m and 4 dS/m respectively. The uncertainties occurred in global climate will influence the performance of crop yield in the future and jeopardizing food security of world's population (Salleh *et al.*, 2018).

Moreover, severe flooding had caused damage to seasonal and unseasonal crop especially rice. Complete submergence during vegetative growth stages especially at seedling or tillering stage may be fatal for the rice plants (Oladosu *et al.*, 2020). Singh *et al.* (2017) however stated that tolerant genotype may survive up to 14 days and will continue growing after the flood subside with little damage to plant morphology. The tolerant genotype might apply two types of tolerant strategies particularly quiescence and escape (Luo *et al.,* 2011). In the quiescence strategy, the plant would conserve energy and carbohydrate consumption thus limit the elongation of shoot and overall growth. In contrast, in the escape strategy, plant would increase shoot elongation in order to escape from the submergence. However, shoot elongation will consume a lot of energy and is not reversible. Hence, after flood subsided, the elongated plant prone to lodging (Luo *et al.*, 2011; Mohd Ikmal *et al.*, 2020). Oladosu *et al.*, (2020) highlighted that the plant may escape submergence stress by lengthening their internode under complete submergence. Iftekharuddaula *et al.* (2019) also stated that complete submergence would affect growth and yield of rice.

Xu and Mackill (1996) reported that the most remarkable advancement in submergence tolerant breeding was the discovery and identification of SUBMERGENCE 1 (*Sub1*) quantitative trait loci (QTL) which confers tolerance to complete submergence. Iftekharuddaula *et al.* (2019) unraveled that *Sub1* is associated with an ethylene responsive factor like mechanism and there are three putative genes in this QTL which are *Sub1*A, *Sub1*B and *Sub1*C. In general, *Sub1* will be up-regulated when exposed to complete submergence and will be down-regulated once the flood subsides. *Sub1* has been been originally mapped and identified from traditional variety called FR13A. Recently, *Sub1* has been transferred to various mega- and elite-varieties of rice such as IR64, Swarna and Samba Mahsuri (Oladosu *et al.,* 2020). On the other hand, Waziri *et al.* (2016) explained that QTL mapping has contributed to the development of salinity tolerant varieties. The major QTL responsible for salinity tolerant has been mapped on chromosome 1 in an F8 Recombinant Inbred Line (RIL) obtained by a cross between Pokkali (salt tolerant) and IR29 (salt sensitive) at the International Rice Research Institute (IRRI) in their salt stress tolerance breeding program (Mohammadi-Nejad *et al.*, 2008). Previous study by Rahman *et al.*, (2019) indicated that *Saltol* QTL was responsible for shoot Na^+/K^+ ration hence conferring salinity tolerance at seedling stage.

Above all, saline water submergence could be regarded as an emerging problem in rice cultivation. The flood is caused by the intrusion of brackish water into rice fields located nearby or along coastal areas. As for now, tolerant genotype to saline water submergence has not been yet reported. Hence, this study was conducted as an attempt to screen for potentially tolerant genotype to saline water submergence from the Malaysian rice cultivars planted by local rice farmers. In addition, IR64-*Sub1* was used as submergence tolerant check, and Pokkali as salinity tolerant check while the IR64 as susceptible check in the present study.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was conducted at Glasshouse and Nursery Complex, Kulliyyah of Science, International Islamic University Malaysia. There were six genotypes of involved in this study mainly MR253, MR284, MR297, Pokkali, IR64 and IR64-*Sub1*. Pokkali and IR64-*Sub1* were control for salinity and submergence tolerant, respectively. The seeds of genotypes were acquired from the National Rice Genebank, Malaysia Agriculture Research and Development Institute (MARDI) while IR64-*Sub1* was acquired from Dr. Noraziyah Abd Aziz Shamsudin from Universiti Kebangsaan Malaysia (UKM). The experiment was conducted using Split-Plot Design with three replication. The main plot was salinity submergence treatment while the sub-plot was rice genotypes.

Planting Procedure

All seeds were pre-germinated in a petri dish by soaking the seed for 24 hours following Salleh *et al.* (2020a). Then, the seeds were transferred onto wet tissue in a petri dish until coleoptile emerged and elongated. The perforated seedling trays size 5cm (length) x 36cm (width) x 6cm (hole diameter) x 4.5cm (depth) were filled with topsoil (Salleh *et al.*, 2020b). Then, the seedlings at the age of 7 days old were transplanted into the tray from petri dish. The seedling trays were placed in the Polyethylene (PE) tank with 2-meter height. The seedlings were grown in the normal condition for 14 days.

Saline Water Submergence Treatment

The seedlings were grown in the normal condition for 14 days. After 14 days, the seedlings were imposed to saline water submergence for the two weeks duration. The polyethylene (PE) tanks were filled with water until the seedlings were completely submerged. There were 4 salinity levels mainly 0 dS/m, 4 dS/m, 8 dS/m and 12 dS/m. The non-submerged seedlings served as control of the experiment and grew control condition that was in normal environment. Regular monitoring of salinity level was done by using portable EC meter namely (COMBI 5000 multiprobe pH+EC).

Data Collection

The parameters mainly plant height, plant height increment and survival rate were collected before submergence, right after de-submerged (RAD), and 14 days after de-submerged (14DAD). In addition, phenotypic scoring was also conducted following the Scoring Evaluation System (SES) established by International Rice Research Institute (IRRI) as shown in Table 1 (IRRI, 2002). The score 1 indicated highly tolerant while score 9 indicated highly sensitive.

Table 1. The Standard Evaluation System Released by IRRI for submergence scoring.

Score	Tolerance Level	Observation
1	Highly Tolerant	High satisfactory growth and tillering, erect dark green leaves
3	Tolerant	Satisfactory growth, erect dark green leaves.
5	Moderately Tolerant	Normal satisfactory growth, erect dark leaves.
7	Sensitive	Low growth and tillering, droopy pale green leaves.
9	Highly Sensitive	Almost all plants dead or dying.

The survival rate of the seedlings was recorded on day 14 after de-submerged following formula by Mohd Ikmal *et al.* (2020):

$$\text{SR (\%)} = \frac{\text{Number of seedlings survived after submergence}}{\text{Number of seedlings planted before submergence}} x100\% \qquad \text{Equation 1}$$

Data Analysis

All data collected were analyzed using the two-way analysis of variance (ANOVA) at $p < 0.05$ followed by Duncan new multiple range test (DNMRT) for mean comparison analysis using the Statistical Analysis System, SAS software.

RESULTS AND DISCUSSION

Based on Table 2, Pokkali recorded as the highest plant height after 14 days of de-submerged (PH-14DAD) at 90.38 cm whereas the rest had almost similar PH-14DAD ranging from 54 cm to 60 cm. Next, Pokkali recorded significantly higher plant height before treatment (PHB) at 76.23 cm as compared to other genotypes under control where non-submerged seedlings grew in normal environment. In contrast, the PHB of all genotypes were not significantly different among each other except for MR297 under Control. This might be because of those genotypes were rice cultivars which have been selected for a semi-dwarf trait during breeding process. Hence, those genotypes recorded not significantly different in terms of plant height trait. As for plant height right after de-submerged (PH-RAD) and PH-14DAD, Pokkali recorded significantly higher plant height at 76.23 cm and 90.38 cm as compared to other genotypes. It is commonly known that Pokkali is a traditional genotype thus having higher plant height as compared to modern rice cultivars.

Table 2. The results of plant height before treatment (PHB), plant height right after de-submerged (PH-RAD), plant height after 14 days of de-submerged (PH-14DAD), plant height increment right after de-submerged (PHI-RAD), plant height after 14 days of de-submerged (PHI-14DAD) and survival rate (SR) of genotypes in respective treatment.

Treatment	Genotype	PHB (cm)	PH-RAD (cm)	PH-14DAD (cm)	PHI-RAD (cm)	PHI-14DAD (cm)	SR (%)
Control	MR 253	22.67^{b} ± (1.86)	47.33^{b} ± (2.07)	54.67^{b} ± (1.03)	24.66^{bc} ± (0.59)	32.00^{a} ± (1.59)	NA NA
	MR 284	25.33^{b} ± (0.19)	55.67^{b} ± (1.41)	60.49^{b} ± (3.25)	30.34^{ab} ± (1.31)	35.16^{a} ± (3.24)	NA NA
	MR 297	15.62^{c} ± (2.65)	51.48^{b} ± (3.93)	54.37^{b} ± (0.75)	35.86^{a} ± (4.34)	38.75^{a} ± (2.97)	NA NA
	Pokkali	76.23^{a} ± (5.00)	76.23^{a} ± (5.00)	90.38^{a} ± (13.88)	0.00^{d} ± (0)	14.15^{b} ± (17.74)	NA NA
	IR64	24.22^{b} ± (0.97)	51.61^{b} ± (2.44)	58.78^{b} ± (3.23)	27.39^{bc} ± (3.39)	34.56^{a} ± (3.06)	NA NA
	IR64-*Sub1*	27.67^{b} ± (0.88)	49.84^{b} ± (1.13)	56.63^{b} ± (3.90)	22.17^{c} ± (1.93)	28.96^{a} ± (3.45)	NA NA
0 dS/m	MR 253	13.33^{b} ± (0.51)	18.64^{c} ±(1.34)	0.00^{b} NA	5.31^{b} ±(1.15)	0^{b} ±(0.00)	0^{c} ±(0.00)
	MR 284	13.67^{b} ±(1.50)	29.48^{ab} ±(1.94)	4.47^{b} ± (2.42)	15.81^{a} ±(2.70)	0^{b} ±(0.00)	17^{b} ±(8.33)
	MR 297	15.62^{b} ±(2.65)	20.71^{c} ±(2.90)	2.68^{b} ± (1.54)	5.09^{b} ±(2.68)	0^{b} ±(0.00)	17^{b} ±(8.33)
	Pokkali	24.44^{a} ±(8.33)	34.81^{a} ±(1.36)	6.14^{b} ± (6.14)	10.37^{ab} ±(2.54)	0^{b} ±(0.00)	8^{b} ±(4.33)
	IR64	15.28^{b} ±(0.70)	23.54^{bc} ±(2.53)	0.00^{b} ± (0.00)	8.26^{ab} ±(2.98)	0^{b} ±(0.00)	0^{c} ±(0.00)
	IR64-*Sub1*	19.28^{ab} ±(2.79)	25.34^{bc} ±(1.36)	30.28^{a} ± (4.70)	6.06^{b} ±(3.63)	11.00^{a} ± (4.90)	83^{a} ±(8.33)
4 dS/m	MR 253	13.33^{b}	31.03^{a}	0.00	17.70^{a}	NA	0^{a}

		±(0.51)	±(1.33)	± (0.00)	±(0.82)	NA	±(0.00)
	MR 284	13.67[b]	39.07[a]	0.00	25.40[a]	NA	0[a]
		±(1.51)	±(1.13)	± (0.00)	±(2.64)	NA	±(0.00)
	MR 297	15.62[b]	37.62[a]	0.00	22.00[a]	NA	0[a]
		±(2.65)	±(9.30)	± (0.00)	±(7.51)	NA	±(0.00)
	Pokkali	24.44[a]	47.8[a]	0.00	23.36[a]	NA	0[a]
		±(3.70)	±(6.95)	± (0.00)	±(4.60)	NA	±(0.00)
	IR64	15.28[b]	33.83[a]	0.00	18.55[a]	NA	0[a]
		±(0.70)	±(0.62)	± (0.00)	±(0.94)	NA	±(0.00)
	IR64-*Sub1*	19.00[ab]	34.04[a]	0.00	15.04[a]	NA	0[a]
		± (2.52)	± (1.39)	± (0.00)	± (1.67)	NA	±(0.00)
8 dS/m	MR 253	19.06[b]	26.41[b]	0.00	7.35[ab]	NA	0[a]
		± (0.56)	± (2.81)	± (0.00)	±(2.25)	NA	±(0.00)
	MR 284	21.67[b]	31.26[b]	0.00	9.59[ab]	NA	0[a]
		±(0.19)	±(1.97)	± (0.00)	±(1.78)	NA	±(0.00)
	MR 297	18.53[b]	28.14[b]	0.00	9.61[ab]	NA	0[a]
		±(0.80)	±(1.24)	± (0.00)	±(2.04)	NA	±(0.00)
	Pokkali	33.06[a]	50.03[a]	0.00	16.97[a]	NA	0[a]
		±(1.69)	±(3.72)	± (0.00)	±(2.10)	NA	±(0.00)
	IR64	17.89[b]	30.41[b]	0.00	12.52[ab]	NA	0[a]
		±(1.56)	±(1.60)	± (0.00)	±(2.01)	NA	±(0.00)
	IR64-*Sub1*	19.00[b]	18.76[b]	0.00	NA	NA	0[a]
		± (0.92)	± (7.88)	± (0.00)	NA	NA	±(0.00)
12 dS/m	MR 253	13.33[b]	14.33[a]	0.00	1.00	NA	0[a]
		±(0.51)	±(1.95)	± (0.00)	±(1.45)	NA	±(0.00)
	MR 284	13.67[b]	18.59[a]	0.00	4.92	NA	0[a]
		±(1.50)	±(0.79)	± (0.00)	±(1.49)	NA	±(0.00)
	MR 297	15.62[b]	12.64[a]	0.00	NA	NA	0[a]
		±(2.65)	±(1.81)	± (0.00)	NA	NA	±(0.00)
	Pokkali	24.44[a]	17.94[a]	0.00	NA	NA	0[a]
		± (3.70)	± (6.03)	± (0.00)	NA	NA	±(0.00)
	IR64	15.28[b]	13.47[a]	0.00	NA	NA	0[a]
		±(0.70)	±(0.27)	± (0.00)	NA	NA	±(0.00)
	IR64-*Sub1*	17.00[b]	14.87[a]	0.00	NA	NA	0[a]
		±(0.58)	±(3.94)	± (0.00)	NA	NA	±(0.00)

NA-not applicable; Values ± of the standard error of mean.

Under 0 dS/m, the mean square values of the analysis of variance of plant height before treatment (PHB), plant height right after de-submerged (PH-RAD), plant height increment (PHI) and survival rate (SR) are shown as in Table 3. The PH-RAD and SR were highly significant at $p<0.01$, while PHB and PHI were not significantly different among genotypes. Based on Table 2, Pokkali recorded significantly higher PH-RAD at 34.81 cm as compared to other genotypes except MR284 (29.48 cm). The IR64-*Sub1* however recorded significantly higher PH-14DAD at 30.28 cm as compared to other genotypes including Pokkali (6.14 cm). For PHI-RAD, MR284 recorded significantly higher plant height increment at 15.81 cm along with Pokkali (10.37 cm) and IR64 (8.26 cm) as compared to other genotypes. The IR64-*Sub1* (6.06 cm) on the other hand recorded statistically similar PHI-RAD with MR253 (5.31 cm) and MR297 (5.09 cm), respectively. Interestingly, the IR64-*Sub1* recorded significantly higher PHI-14DAD at 11.00 cm as compared to other genotypes. In fact, other genotypes recorded new shoot growth thus there were no increment in plant height on PHI-14DAD. All genotypes recorded high PHI-RAD because the plants tried to adapt with complete submergence by elongating their shoot and stem (Luo *et al.*, 2011). However, after recovery phase at PHI-14DAD, the elongated stem was

irreversible thus those plants were prone to lodging after flood water subsides as studied by Luo *et al.* (2011) and Mohd Ikmal *et al.* (2020).

Table 3. Analysis of variance (mean square values) for plant height before submergenced (PHB), plant height right after de-submerged (PH-RAD), plant height increment (PHI) and survival rate (SR) under 0 dS/m.

Source of variation	df	PHB	PH-RAD	PHI	SR
Genotypes	5	53.98^{ns}	105.80**	3116.88^{ns}	2974.22**
Blocks	2	2.86^{ns}	3.59*	5.90^{ns}	342.72*
Error	10	18.31	13.67	1447.48	67.72

*significant at $p<0.05$, **significant at $p<0.01$

In addition, under 0 dS/m, the SR of IR64-*Sub1* was significantly higher at 83% as compared to other genotypes mainly MR284 (17%), MR297 (17%), Pokkali (8%), IR64 (0%), and MR253 (0%). Moreover, based on the IRRI's Standard Evaluation System (SES) scores (The Standard Evaluation System, 2002), IR64-*Sub1* recorded as score 3 based on Table 1. which indicated higher tolerant ability as compared to other genotypes mainly MR284, MR297 and Pokkali which recorded SES score at 7 and MR253 and IR64 at score 9 as shown in Table 4. As record, MR253 and IR64 were found to be highly susceptible to submergence at 0 dS/m. Nonetheless, the addition of *Sub1* QTL into IR64 significantly improve SR under fresh water submergence at 0 dS/m as shown by the IR64-*Sub1* in the present study. Previous study by Septiningsih *et al.*, (2009) also indicated that rice genotypes with *Sub1* QTL recorded significantly higher SR as compared to genotypes without *Sub1* QTL. The *Sub1* QTL was also reported to be associated with the transcription factor for ethylene response factor (ERF) (Xu *et al.*, 2006). Submergence stress would induce the accumulation of ethylene in the plant. Increasing amount of ethylene in the rice plant under submergence would later trigger the down-regulated of the Gibberallic acid (GA) production in plant with *Sub1* QTL. As result, shoot elongation under submergence will be limited as shown in the PHI-RAD of IR64-*Sub1* in the present study. Furthermore, the presence of *Sub1* QTL had conserved the plant energy by lowering down carbohydrate consumption in order to prevent chlorophyll breakdowns thus diverged the energy pathway simultaneously (Emerick & Ronald, 2019).

In contrast, under saline water submergence at 4, 8, and 12 dS/m, there was no genotypes were survived including tolerant IR64-*Sub1* and Pokkali. According to Rumanti *et al.* (2018), Pokkali may survive under salinity condition at 12 dS/m due to the presence of salinity tolerant QTL called *Saltol* but was found to be susceptible to submergence stress. The IR64-*Sub1* on the other hand may survive under fresh water submergence but was found to be susceptible to salinity stress. Hence, based on results of the present study, it might be speculated that saline water submergence probably involves a novel QTL or genes, and different tolerant mechanism as compared to fresh water submergence (*Sub1* QTL) and salinity stress (*Saltol* QTL) per se. Since there were yet any report on the QTL associated with tolerant ability to saline water submergence, further study hence needed to screen and identify potential donor from rice germplasms originated from coastal area.

Table 4. The results of Standard Evaluation System (SES) of genotypes in respective treatment after 14 days of de-submerged (14DAD) in different treatments (saline water submergence).

Treatment	Genotype	SES
Control	MR 253	1
	MR 284	1
	MR 297	1
	Pokkali	1
	IR64	1
	IR64-*Sub1*	1
0 dS/m	MR 253	9
	MR 284	7
	MR 297	7

	Pokkali	7
	IR64	9
	IR64-*Sub1*	3
4 dS/m	MR 253	9
	MR 284	9
	MR 297	9
	Pokkali	9
	IR64	9
	IR64-*Sub1*	9
8 dS/m	MR 253	9
	MR 284	9
	MR 297	9
	Pokkali	9
	IR64	9
	IR64-*Sub1*	9
12 dS/m	MR 253	9
	MR 284	9
	MR 297	9
	Pokkali	9
	IR64	9
	IR64-*Sub1*	9

NA-not available

CONCLUSION

In conclusion, all genotypes were susceptible to saline water submergence at 4, 8, and 12 dS/m. However, under fresh water flood at 0 dS/m, IR64-*Sub1* recorded significantly higher survival rate at 83% as compared to other genotypes. Hence, IR64-*Sub1* may be promoted to local rice farmers with fresh water flood problem. Further screening on rice genotypes collected from coastal areas thus needed in order to identify potentially tolerant genotype to saline water submergence. Alternatively, combining *Sub1* and *Saltol* QTLs via marker-assisted breeding might probably produce potentially tolerant lines to saline water submergence.

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