EVALUATION OF SELECTED RICE MUTANT GENOTYPES TOLERANT TO SUBMERGENCE AT GERMINATION AND SEEDLING STAGE

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ABSTRACT

Submergence is one of the major abiotic stresses that limits rice productivity. With climate change expected to become more extreme shortly, the frequency of submergence is also expected to increase. Therefore, the development of new submergence-tolerant rice varieties is necessary as one of the best environmentally friendly solutions to overcome this problem. In this study, three selected mutant rice genotypes developed by Malaysian Nuclear Agency (MNA) through gamma-ray, together with one submergence tolerant and one submergence susceptible rice genotypes were screened under anaerobic germination (AG) and vegetative stage submergence stress (VS). This experiment was conducted using a randomized completely block design (RCBD) with three replications. For AG, all seeds were germinated in the test tube while the water level was maintained at 18 cm for 21 days in dark condition. Elongation (ED) was measured on a daily basis while germination percentage (GP) and survival rate (SRG) were collected after 10 days after stress imposition and 10 days after de-submerge respectively. For VS, 21 days-old seedlings were fully submerged for 14 days. Data on plant height before (PHB) and after (PHA) submergence, elongation percentage (EP), and survival rate (SRS) were recorded in VS screening. Analysis of variance (ANOVA) showed significant *differences among rice genotypes for survival rate in both AG and VS experiments. Results show a high variation of GP (53.33 to 100%), ED (0.10 to 0.27), SRG (40 to 93.30%) PHB (15.87 to 24.27 cm), PHA (19.53 to 32.67 cm), EP (10.57 to 92.40%) and SRS (20 to 86.67%). One mutant genotype, NMR 151 showed highest SRG during AG (93.30%) compared to tolerant check, IR64 sub-1 (86.67%). Moreover, both mutant genotypes which are, NMR 151 and NMR 152 showed higher SRS during vegetative stage (NMR 151: 66.67% and NMR 152:73.33%) compared to susceptible check, MR 219 (20%), but lowest compared with check variety, IR64 sub-1 (86.67%). Thus, mutation breeding is one of the breeding techniques efficient to develop submergence tolerant rice in this modern era.*

Key words: *Oryza sativa*, rice mutant lines, abiotic stress, submergence tolerant

INTRODUCTION

Submergence is one of the abiotic factors limiting rice production in Malaysia. Major total lost was facing by our rice farmers due to flooding in Rompin, Pahang (Mamat, 2019). The incidence of the flood in Malaysia showed an increasing trend due to climate change, urbanization, and land overuse (Yusoff, Ramli, Alkasirah, & Nasir, 2018). Additionally, there are 3 types of flood which are submergence during germination, flash flood and stagnant flooding (Reddy et al., 2015). Many farmers in other country have been planting the rice variety adaptable and withstand in submergence condition up to 10 days (Muhammad Shafie M.S, 2018). In Malaysia, lacking the tolerant submergence rice variety is one of the problems facing by our rice industry player. Introduction of a new rice variety adaptable in submergence tolerance is highly recommended.

Many of mega-variety developed in Malaysia are absent gene responsible for submergence tolerant. Submergence tolerance is controlled by single gene *sub-1* which is mapped from landrace variety, FR13A (Ahmed et al., 2016). Many improved submergence tolerant rice varieties in the world have been introduced with *sub-1* gene such as Swarna *sub-1* and IR64 *sub-1*. Moreover, high carbohydrate concentration, preservation high chlorophyll in leaf, and regeneration after submergence are the crucial traits responsible for submergence tolerant rice variety (Oladosu et al., 2020). Thus, evaluation of high yielding rice genotypes derived from the breeding program to submergence tolerant is crucial to select promising genotypes to be introduced in flood prone-area.

Mutation breeding is one of the methods for developing new plant varieties in seed and vegetative propagated. This method widely used by breeder over the world in this era. The advantage is the stable new mutant varieties could be developed in a quick and short time compared with conventional method respectively (Shu, Forster, Nakagawa, & Nakagawa, 2012). In Malaysia, a few potential rice mutant genotypes with good agronomical traits and high yielding have been developed by Malaysian Nuclear Agency (MNA). Unfortunately, no study has been done for submergence tolerance adaptability for these mutants. Evaluation of submergence tolerant traits will give a value-added and new knowledge for these mutant genotypes as well as the potential genotypes introduced in flood-prone area. Thus, the objective of this research is (i) to screen and select the potential rice mutant genotypes tolerant under anaerobic germination (AG) and vegetative stage submergence (VS).

METHODOLOGY

Plant materials

Five rice genotypes were obtained from Malaysian Nuclear Agency (MNA), Malaysian Agricultural Research and Development Institute (MARDI) and Universiti Kebangsaan Malaysia (UKM). The list of the genotypes as shown in Table 1.

Table 1: List of rice genotypes

No	Genotypes	Status	Source
1.	NMR 151	Mutant line	MNA
2.	NMR 152	Mutant line	MNA
3.	MA03	Mutant line	MNA
4.	MR 219 (Submergence susceptible)	Breeding line	MARDI
5.	IR64 *Sub-1* (Submergence tolerant)	Breeding line	UKM

Experimental design and layout

Five rice genotypes used in this experiment were screened under anaerobic germination (AG) and vegetative stage (VS) submergence stress. The experiment design was randomized completely block design (RCBD) with three replications. For AG, all the rice genotypes were germinated under the dark condition with 18 cm water level for 21 days in the test tube. The data collected are: (i) Elongation per day (ED)(cm), (ii) germination percentage (GP) (%), (iii) and survival rate (SRG) (%). For VS, 21 days of healthy seedling for all 5 genotypes were fully submerged for 14 days under 80 cm water level. The data collected are: (i) plant height before submergence (PHB) (cm), (ii) plant height after submergence (PHA) (cm), (iii) elongation percentage (EP) (%) and (iv) survival rate (SRV) (%).

Data analysis

All the data collected were subjected to Analysis of Variance (ANOVA) using Statistical Tool for Agricultural Research (STAR) version 2.0.1.

RESULTS

Survival evaluation of anaerobic germination

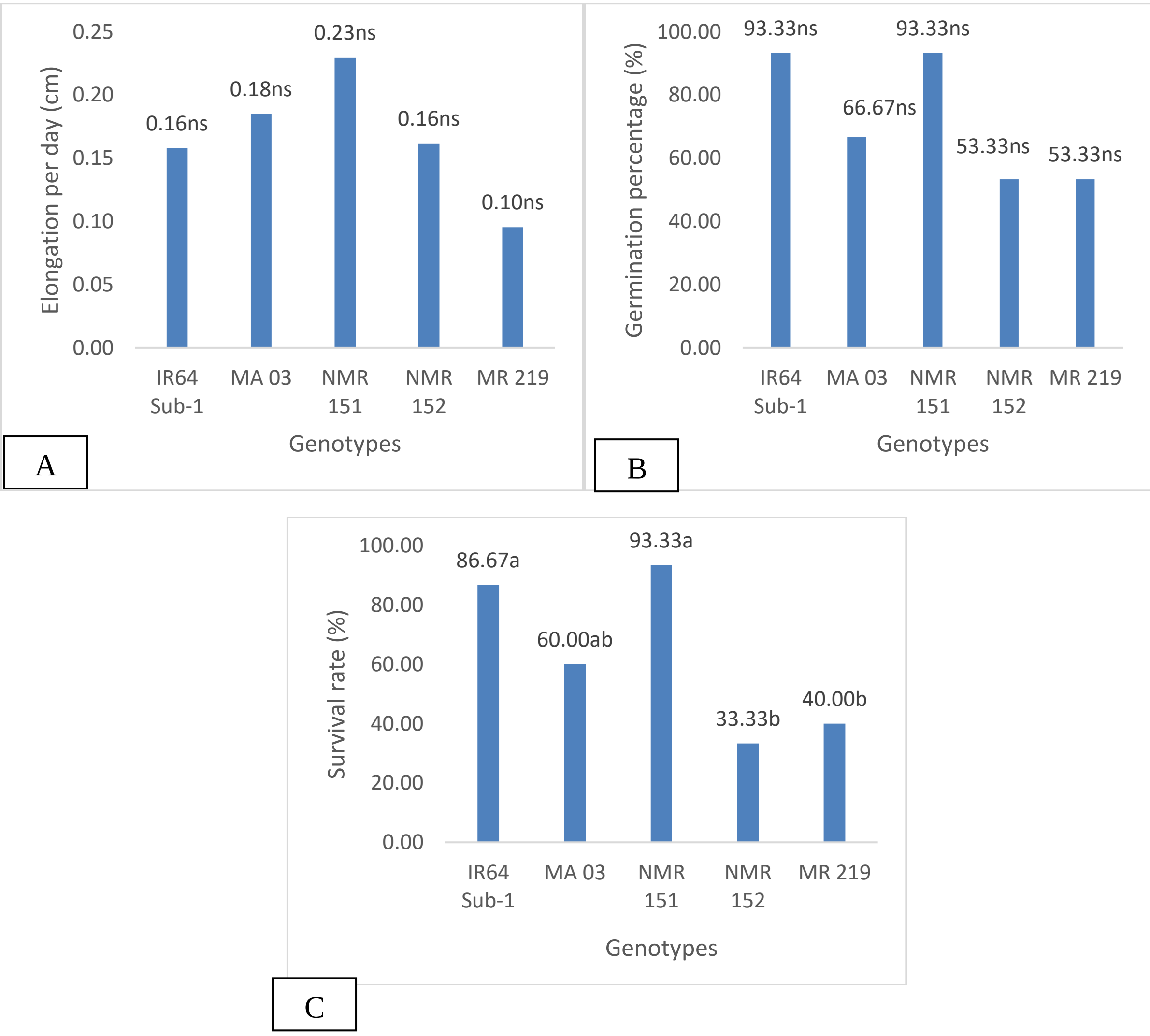
Survival rate (SRG) showed a significant difference at ($p \leq 0.05$) among all genotype tested (Table 2). Additionally, mutant rice line NMR 151 showed the highest SRG followed by submergence tolerant genotype IR6-*Sub1* (Figure 1C). The results also showed high variation of GP (53.33 to 100%) and ED (0.10 to 0.27cm), respectively (Figure 1A and IB). Highest ED (0.23 cm) and GP (93.33%) also recorded by most tolerant genotype, MNR 151 (Figure 1A and 1B).

Table 2: Mean squares of analysis of variance for 3 characteristics among 5 rice genotypes under anaerobic germination

Source	Df	ED	GP	SRG
Block	2	0.0046^{ns}	240.0000^{ns}	746.6667^{ns}
Genotypes	4	0.0070^{ns}	1226.6667^{ns}	2173.3333*
Error	8	0.0054	606.6667	513.3333

Note: Df-degree of freedom, ED-elongation per day, GP-germination percentage, SRG-percentage of survival rate
*-Significant at 0.05 level
ns- not significant

Figure 1: Data collected for Anaerobic Germination (AG). (A) elongation per day, (B) germination percentage (%), (C) percentage of survival rate (%)



Note: means with different letter column are statistically different among treatment by LSD test ($p \leq 0.05$).

Survival evaluation of vegetative stage submergence

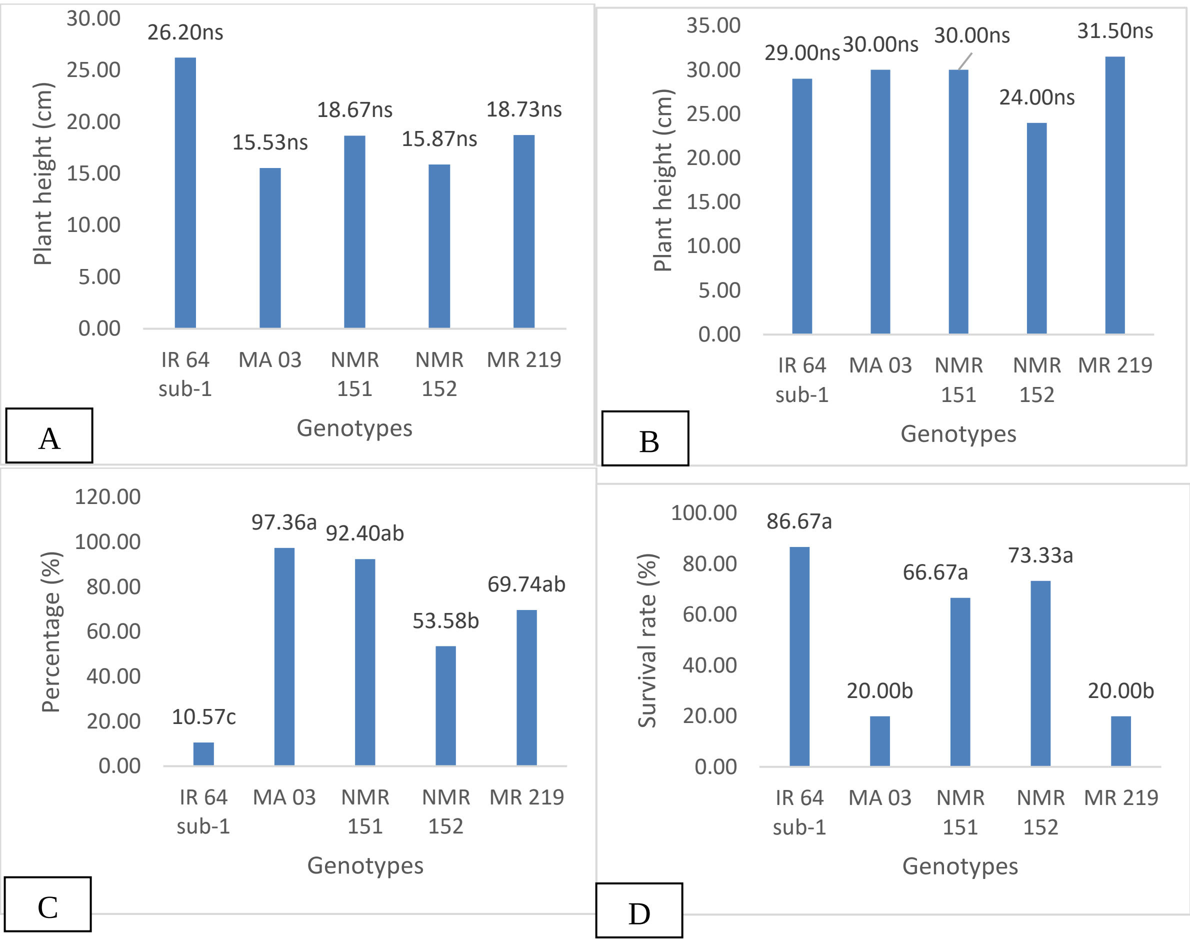
Elongation percentage (EP) and SRG showed a significant difference at (p≤0.05) among all genotype tested (Table 3). The results also showed high variation of PHB (15.87 to 24.27 cm), PHA (19.53 to 32.67 cm), EP (10.57 to 92.40%) and SRS (20 to 86.67%) under VS (Figure 2). Positive tolerant check genotype, IR64 *sub-1* showed higher SRG (86.67%) among the genotypes. Two rice mutant genotypes, NMR 151 and NMR 152 showed higher SRG compared to susceptible genotypes, MR 219.

Table 3: Mean squares of analysis of variance for 4 characteristics among 5 rice genotypes under vegetative stage submergence

Source	Df	PHB	PHA	EP	SRS
Block	2	4.5680^{ns}	5.4500^{ns}	41.7379ns	106.6667ns
Genotypes	4	55.3933^{ns}	24.9000^{ns}	2992.7773*	2933.3333*
Error	8	14.8813	13.3250	481.6098	173.3333

Note: Df-degree of freedom, PHB-plant height before submergence, PHA-plant height after submergence, EP-elongation percentage (%), SRG-percentage of survival rate
*-Significant at 0.05 level
ns- not significant

Figure 2: Data collected for vegetative stage submergence (VS). (A) plant height before submergence, (B) plant height after submergence, (C) elongation percentage (%), (D) percentage of survival rate (%)



Note: means with different letter column are statistically different among treatment by LSD test (p≤0.05).

DISCUSSION

Anaerobic germination and vegetative stage submergence are the types of flooding that occurs in the rice production system (Singh, Septiningsih, Balyan, Singh, & Rai, 2017). The ability of the rice genotypes adaptable in both conditions can reduce total loss by the farmers. From the AG screening results, one mutant genotype, NMR 151 had a high survival rate compared to submergence tolerant varieties, IR64 *sub-1*. The germination rate of NMR 151 is also similar to IR64 *sub-1*. These mutant have the potential to be introduced in the flood-prone area which used the direct seeding method. Direct seeding method was preferred by farmers compared transplanting due to the low cost of labour and time (Yamauchi, 1995). Additionally, rice has an ability to grow its coleoptiles under absent of oxygen. Currently, many QTL's related to AG has been identified by many researchers. About four

QTL's linked to anaerobic germination derived from IR64 and Kharsu 80A population was identified in chromosome 7 and 3 (*qAG7.1*, *qAG7.2* and *qAG7.3*, and *qAG3*) (Baltazar et al., 2019).

Positive tolerant check genotype IR64 *Sub-1* showed a lower elongation percentage (10.57%) under VS. Suppression of shoot elongation via the mediation of *sub-1* and cause the energy will be consumed during submergence leads to the increasing of survival rate (Ikmal, Amira, & Noraziyah, 2019). From the results from VS screening, NMR 152 showed the highest survival rate followed by NMR 151, respectively. Both genotypes can be promoted for planting in flood-prone areas in Malaysia. Another mutant rice genotype, MA03 is considered as susceptible since the SRG was similar to susceptible check genotype, MR 219. In addition, NMR 151 can be recommended to be planted under direct seeding condition as it can germinate well under AG. Further study and improvement of these mutant need to be done since both genotypes showed different mechanism under AG and VS condition. Identification of the QTL's will be revealed the genetic dissection of these mutant genotypes.

CONCLUSION

Two mutant rice genotypes, namely NMR 151 and NMR 152 have been potential to be planted under anaerobic germination (AG) and vegetative stress (VS) submergence. Genotypes NMR 151 showed survival rate more than 60% under both AG and VS condition meanwhile genotype NMR 152 in VS condition only. Exploration and identification of new QTL's linked to AG and VS derived both mutant genotypes are highly recommended to identify vital gene influencing its survival.

ACKNOWLEDGEMENT

The authors would like to thanks to the Ministry of Higher Education for providing research grant under FRGS grant (FRGS/1/2019/WAB01/MOSTI/02/1). Special acknowledge to International Atomic Energy Agency (IAEA) for support in term of training and capacity building, Malaysian Nuclear Agency (MNA) and Universiti Kebangsaan Malaysia (UKM) for providing research facilities. Special thanks also to Civil Service Department of Malaysia (JPA) for provided his scholarship under Hadiah Latihan Persekutuan (HLP).

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