Screening of Submergence Tolerant Myanmar Rice Varieties and Their Molecular Marker Survey

Sandar Aung[1], Ei Ei Nyein[2], Aye Aye Mu[3], Htike Htike Linn[4], San San Aye*

ABSTRACT

Submergence tolerance is an importance that where short term flash flooding damages rice. Tolerant landraces that withstand submergence for 2 weeks were identified. This study is to identify the physiological traits of submergence tolerant varieties of rice plants were taken from Gene Bank, Department of Agriculture Research, Ye-Zin, Nay-Pyi-Taw, Myanmar. In the present study, submergence tolerant of 7 rice verities were tested by two methods. Sub 1 locus were studied by molecular marker survey of the Myanmar rice varieties. This study was conducted at University of Yangon and Nagoya University for marker survey. The two varieties, Sit-pwa and Kar-lii-latt-yong are good tolerance of submergence and good germination under anaerobic condition using directed seeds method and Tataung-po is submergence tolerance and grows well under 5cm water in pre-germinating method. Myanmar rice varieties were not possessed the Sub1 A-1 and Sub 1 C-1 combination. Submergence tolerance of the 12 varieties cannot be attributed to the known A-1 C-1 combination.

INTRODUCTION

Rice is one of the most important food crops in the world, being the staple food for almost half of the world population (Khush, 1997). About 60% of the populations in Myanmar are living in rural area and their livelihood is agriculture. Climate change is causing adverse effects on rice production, which is the most important crops. Water submersion can severely affect crop production, since it drastically reduces oxygen availability necessary for plant's respiration. Plant tolerance to flooding evolved morphological, physiological and biological adaptations to oxygen deficiency. Submergence is stressful for higher plants because it inhibits the entry of atmospheric oxygen to plant leaves and roots due to the very slow rate of the diffusion of gases in water. Submergence had a deleterious effect on the ruderal population, which is evidenced by leaf decay and blackening of the root tissue (Lynn & Waldren, 2003). Sub1 gene was reported and cloned as one of the most important genes for rice submergence tolerance. Clone as a gene for submergence tolerance (Xu *et al*., 2006). Sub 1 locus consists of 3 homologous genes, SUB 1 A, SUB 1 B and SUB 1 C. Some non-tolerant varieties lack SUB 1 A. So far, it is known that tolerant varieties possess SUB 1A-1 and SUB 1C-1 alleles. The aim and objectives of this research works are to study the effect of anaerobic condition on the rice plant growth, to evaluate submergence tolerance Myanmar rice varieties, to establish a system for DNA marker survey of Sub 1 for Myanmar rice varieties and to identify the alleles of SUB 1 A and SUB 1C in Myanmar submergence tolerant varieties.

MATERIALS AND METHODS

---

[1] Dr, Lecturer, Department of Botany, Dagon University
[2] Dr, Assistant Lecturer, Department of Botany, University of Yangon
[3] Dr, Assistant Lecturer, Department of Botany, Dawei University
[4] Dr, Assistant Lecturer, Department of Botany, University of Yangon
* Corresponding author, +9595000304, Dr, Pro-Rector, Department of Botany, Mawlamyine University

Rice samples and screening experiment

Submergence tolerance varieties: Sit-pwa, Kauk-hnyin-hmwe, Tataung-po, Hnang-ga, Kar-lii-latt-yong, Shwe-chay-chin and Yadanar-win were taken from Gene Bank, Department of Agriculture Research, Ye-Zin, Nay-Pyi-Taw. A screening experiment to obtain submergence tolerant types of rice was conducted using seven varieties of rice in the wet season of 2015, at Department of Botany, University of Yangon. The seeds of seven rice varieties were tested by two methods to germinate the seeds. The first method was direct seeding in anaerobic environment and another was pre-germinating seeds in room temperature.

Seeds bed preparation

Experiment was carried out according to the randomized complete block design (RCBD) with 4 replicates. The 15 dried seeds of each variety were placed in the seed bed prepared in the plastics trays (20×25×12) cm. Each variety was separated by bamboo to avoid running of seeds. After positioning the seeds in the seed bed, the seeds were covered with soil. The control treatments were kept in the room temperature.

For direct seeding method, the 15 dried seeds of each variety were used four treatments. The plastic trays were submerged 1cm, 2cm, 3cm and 4 cm in water levels respectively. The pre-germinations seeds were submerged 5cm in water levels.

All treatments expect control treatment were submerged for 12 days, adjusted and maintained water level every day.

Preparing seed bed

Covered with soil

Submerged in various water levels

Figure1. Direct seed Method for anaerobic germination

Data collection

Dissolved oxygen is measured one week interval during submergence period of two weeks by using the multimeter Innolab (MULTI-9310). After two weeks, the samples were desubmerged and recorded the germination percentage. The survival rate, and late germination were recorded after 5 days of recovery state.

For pre-germination seeds experiment, the data was recorded after 10 days submerging under 5 cm of water.

For Marker Survey

12 Rice varieties from Myanmar were used as plant materials.

Variety name	Germplasm ID
R-1	000086
R-2	01212
R-3	1250
R-4	2581
R-5	2253
R-6	12007
R-7	00511
R-8	00885
R-9	001160
R-10	00740
R-11	6071
R-12	1524
Biotech-1 (R-10)	00740
Biotech-2 (R-11)	6071
Biotech-3 (R-9)	001160
Biotech-4 (R-4)	2581
IR64	
IR64+Sub1	

DNA was extracted by using CTAB or magnetic beads methods. DNA samples are checked by using electrophoresis by 0.8% agarose gel and quantified with Quantus fluorometer (can detect double strand DNA). PCR primers are based on Singh et al. (2010) (Theoretical and Applied Genetics 121: 1441-1553.)

Recovering plant lysate. Quantus Fluorometer Magnetic beads are added
to the lysate.

Figure 2. DNA Extraction

Primers for SUB1A

GnS2_SUB1 A 203 f cttcttgctcaacgacaacg

GnS2 rev tcgatggggtcttgatctct

Primers for SUB1C

Sub1C 173 f   aacgccaagaccaacttcc

SUB1C 173 r   aggaggctgtccatcaggt

PCR products were digested with *Alu* I (*SUB1A*) or *Cac* 8I (*SUB 1 C*) for discriminating the alleles.

Amplified products and digested products were subjected to electorophoresis (4% agarose gel) to determine the fragment sizes.

RESULTS

Rice samples and Screening Experiment

Germination

Five rice varieties: Sit-pwa, Kauk-hnyin-hmwe, Tataung-po, Hnang-ga and Kar-lii-latt-yong were used by anaerobic germination using direct seeding method. Sit-pwa is the highest percentage of germination rate of 4cm in submerged water level but Sit-pwa survived 100 % at 3 cm submerged water level and the lowest at 1 cm. Hnang-ga and Kar-lii-latt-yong were showed moderately germination rate. Kuak-hnyin-hmwe and Tataung-po cannot germinate under anaerobic condition.

Table 1: Germination rate (%) of five rice varieties under anaerobic condition

Treatments	Sit-pwa	Kauk-hnyin hmwe	Tataung-po	Hnang-ga	Kar-lii-latt-yong
Control	100	100	100	100	100
Treatment-1	46.60	0	0	13.33	13.33
Treatment-2	53.33	0	0	0	0
Treatment-3	60%	0	0	13.33	33.33
Treatment-4	73.33%	0	0	20%	13.33

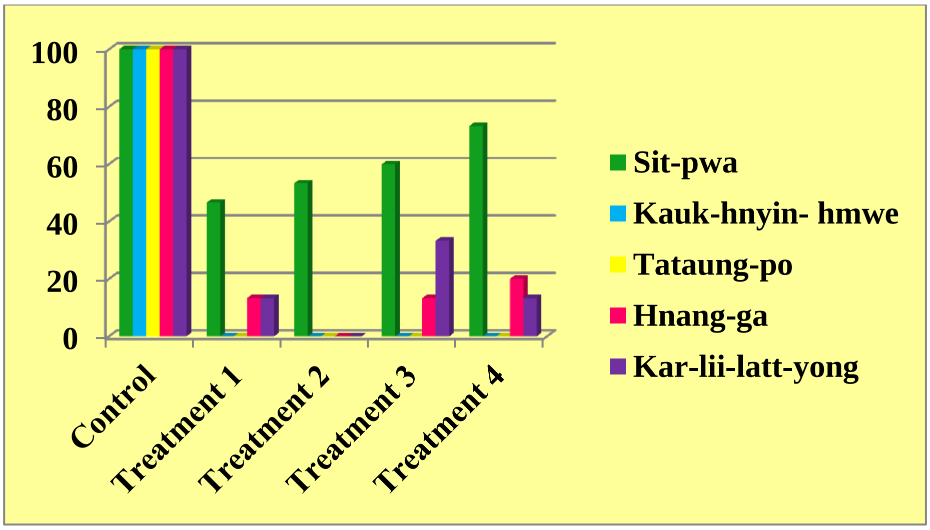


Figure 3. Germination rate (%) of five rice varieties under anaerobic condition

Recovery from submergence stress

After 5 day, the recovery of submergence stress were recored. Sit-pwa survived 100% at 3 cm submerged water level and the lowest at 1 cm, Kauk-hnyin-hmwe and Tataung-po could not germinate no longer, Hang- ga recovered 50% germinated seedling and Kar-lii-latt-yong had the best survival rate at 1 cm and 4 cm. Therefore, these two varieties are showed good tolerance of submergence and germination under anaerobic condition among five varieties.

Plant Height

After desubmerging, Sit-pwa recovered from submergence stress and grew well so that it had the highest plant height of about 17 cm. The rice variety, the average plant heigh of Hnang-ga was 5cm. Kar-lii-latt-Yong survive and grew well so that the average plant height was 7cm in treatment 1, 9 cm in treatment 3 and 6cm in treatment 4.

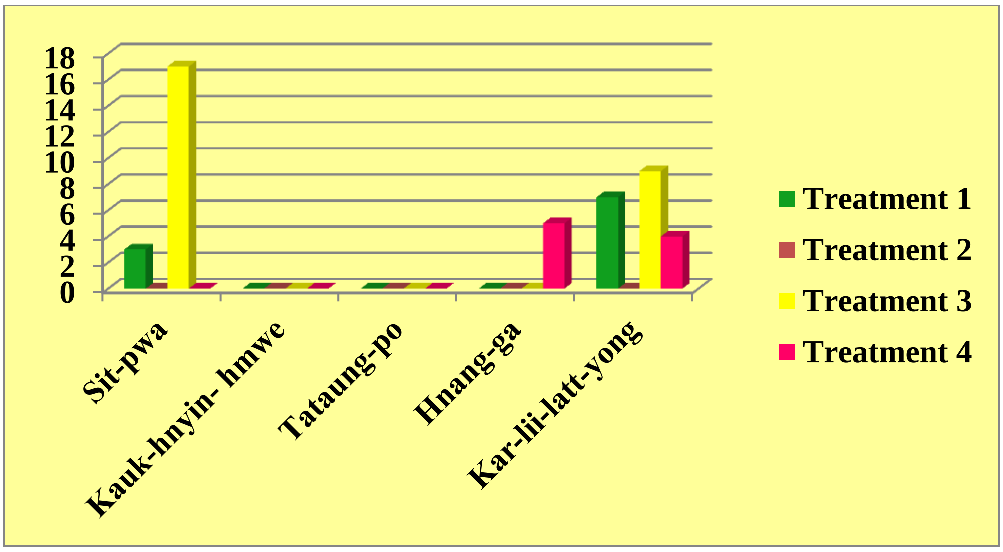


Figure 4. Survival rate (%) of five rice varieties 5 days after desubmergencing

In per-germinating seeds, five varieties; Tataung-po, Shwe-chay-chin (Hmawbi), Kauk-hnyin-hmwe, Shwe-chay-chin and Yadanar-win were used to determine anaerobic germination at 5 cm water level. Yadanar-win was the highest percentage of germination rate, and Shwe-chay-chin (Hmawbi) was the lowest percentage. Tataung-po, Kauk-hnyin-hmwe and Shwe-chay-chin were slightly different in comparison with each other.

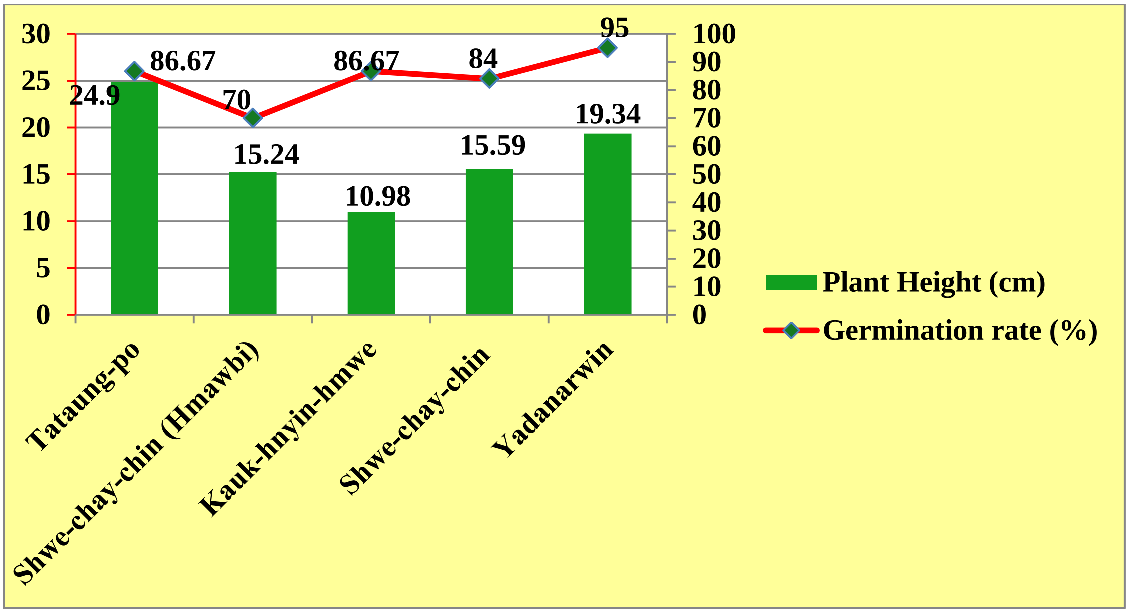


Figure 5. The germination and continuous growth of pre-germinated seeds of five rice varieties

Dissolved oxygen is measured at 3 days and 11 days after submergence. It was found that dissolved oxygen levels in all treatments were reduced during submerging. In 1cm submerged water level, dissolved oxygen was reduced from 6.83 to 5.79, in 2cm, dissolved oxygen was decreased from 7.07 to 6.61, in 3 cm water level, dissolved oxygen was declined from 7.27 to 6.58 and in 4 cm water level, dissolved oxygen was decreased from 7.04 to 6.44.

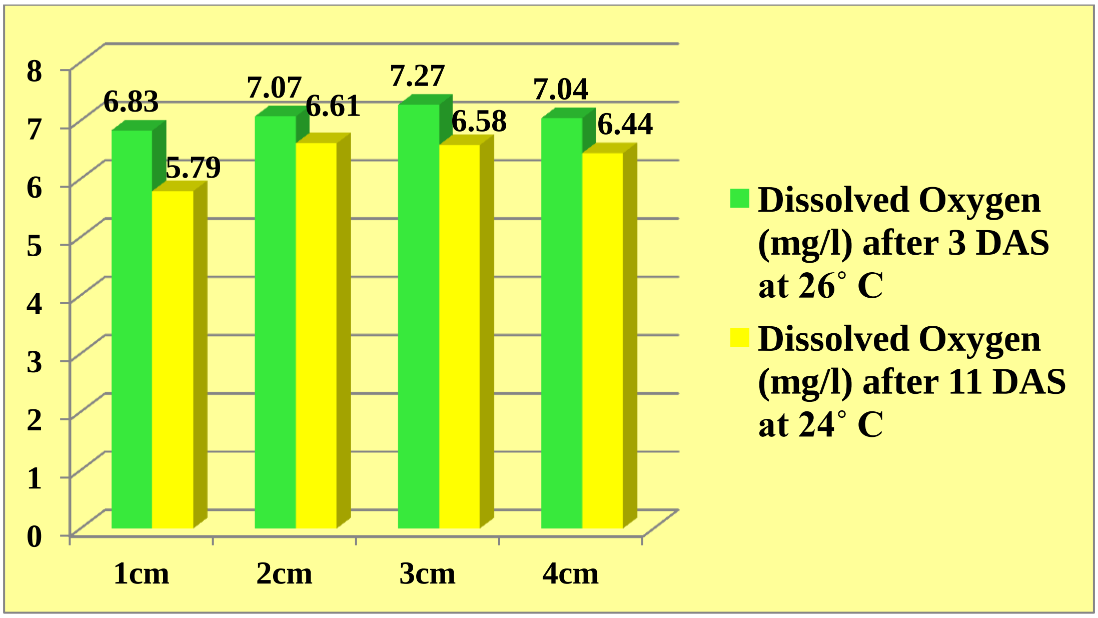


Figure 6. Dissolved oxygen reduction of water

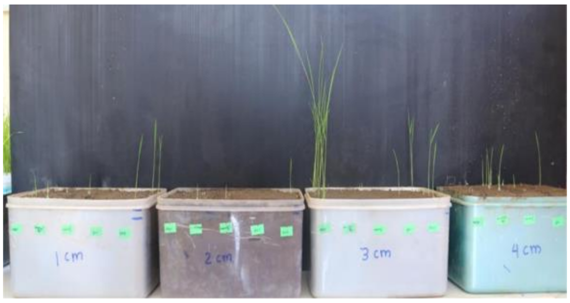


Figure7. Survival rate (%) of five rice varieties 5 days after desubmergenin g

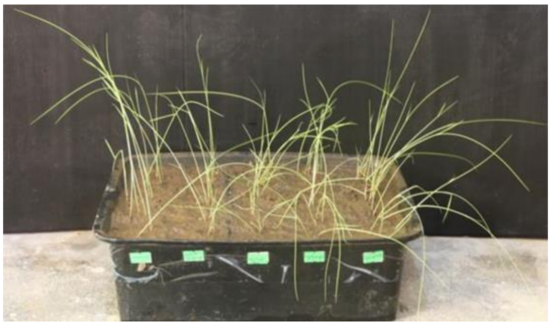
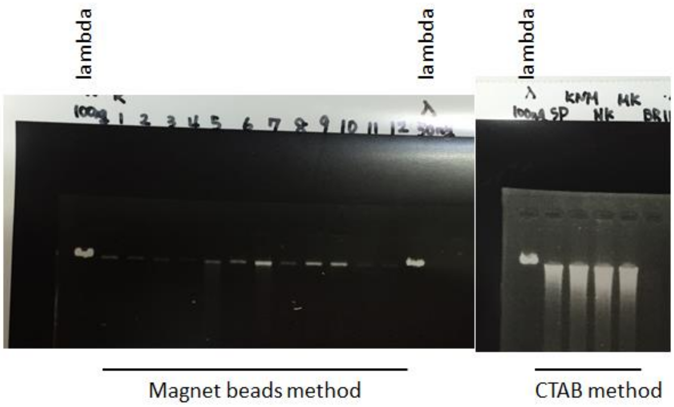


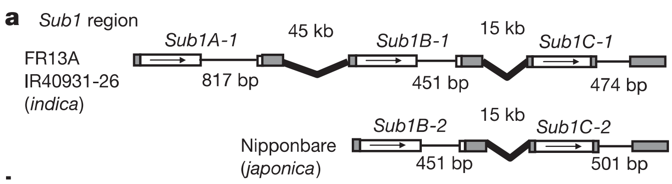
Figure8.The germination and continuous growth of pre-germinated seeds of five rice varieties

For Marker Survey

DNA quality



Detection of SUB1A

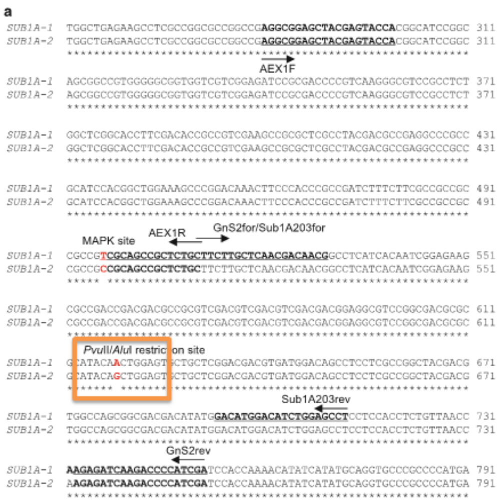


Drought tolerant varieties possess SUB1A. But presence of SUB1A is not sufficient for submergence tolerance.

DNA quantity

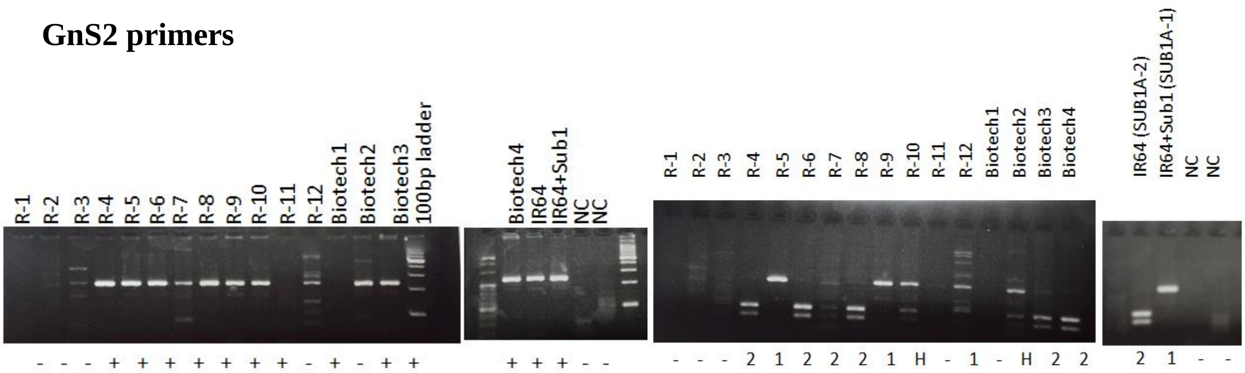
Sample ID	Concentration (ng/uL)
R1	11.0
R2	7.1
R3	9.4
R4	4.1
R5	22.0
R6	11.0
R7	30.0
R8	7.4
R9	14.0
R10	12.0
R11	3.2
R12	1.5
Biotech-1(SP)	105.0
Biotech-2(KNM)	121.0
Biotech-3(NK)	118.0
Biotech-4(MK)	127.0

SUB1A alleles



2 alleles, namely, SUB1A-1 and SUB1A-2 were known. Once amplified by using PCR, these 2 allele can be discriminated with *Alu* I restriction enzyme (-1= not cut, -2= cut)

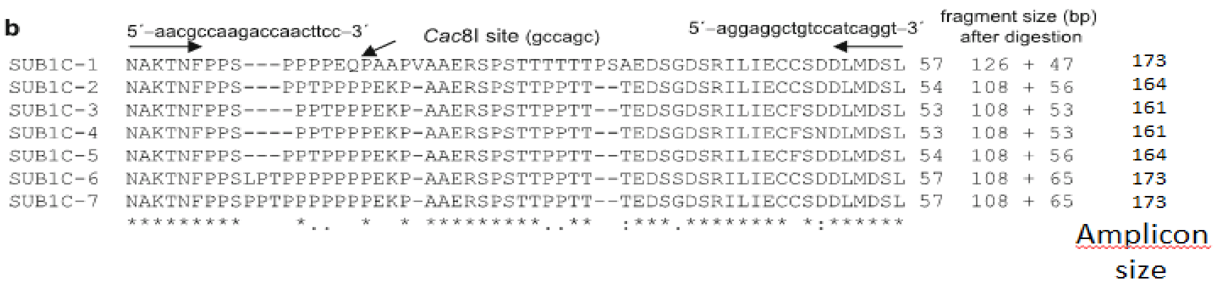
Amplification of SUB1A by using GnS2 primers **Discrimination of SUB1A**



GnS2 primers: 242 bp products were expected. GnS2 PCR products were digested with Alu I.

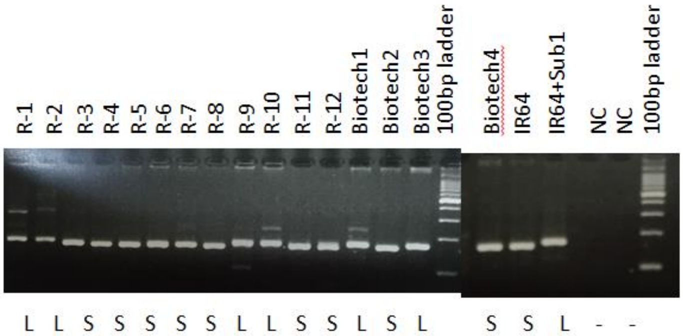
SUB1A-1: not cut (242bp)
SUB1A-2: cut (133bp+109bp)
R-nd R-12: DNA amount may not be sufficient.

Detection of *SUB1C*



SUBIC-is thought to contribute to tolerance.

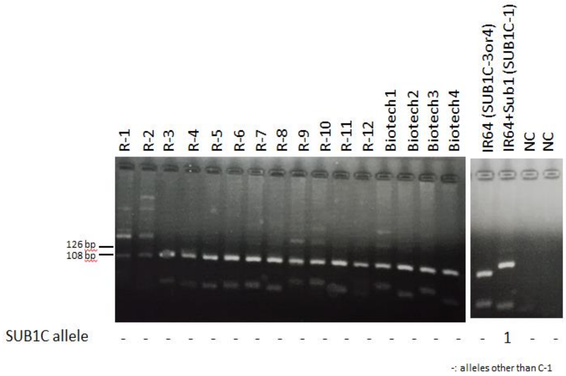
Amplification of *SUB1C* by using SUB1C173 primers



L: 173 bp products (*SUB1C-1*, *6*, or *7*)

S: 164 or 161 bp products (*SUB1C-2, 3, 4* or *5*)

Discrimination of *SUB1C* alleles



SUB1C-1: 126 bp + 47bp

SUB1C-2, 3, 4, 5, 6, 7: 108 bp + (53-65bp)

Table 3. *Sub1* haplotypes of Myanmar rice varieties

Variety name	Germplasm ID	SUB1A amplification	SUB1A SNP	SUB1A allele	SUB1C amplification	SUB1C digested	SUB1C allele
R-1	000086	-	-	-	long	108bp	SUB1C-6/7
R-2	01212	-	-	-	long	108bp	SUB1C-6/7
R-3	1250	-	-	-	short	108bp	SUB1C-2/3/4/5
R-4	2581	+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
R-5	2253	+	A	SUB1A-1	short	108bp	SUB1C-2/3/4/5
R-6	12007	+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
R-7	00511	+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
R-8	00885	+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
R-9	001160	+	A	SUB1A-1	long	108bp	SUB1C-6/7
R-10	00740	+	Hetero?	?	long	108bp	SUB1C-6/7
R-11	6071	-	-	-	short	108bp	SUB1C-2/3/4/5
R-12	1524	+?	G?	SUB1A-2?	short	108bp	SUB1C-2/3/4/5
Biotech-1 (R-10)	00740	-	-	-	long	108bp	SUB1C-6/7
Biotech-2 (R-11)	6071	+	Hetero?	?	short	108bp	SUB1C-2/3/4/5
Biotech-3 (R-9)	001160	+	G	SUB1A-2	long	108bp	SUB1C-6/7
Biotech-4 (R-4)	2581	+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
IR64		+	G	SUB1A-2	short	108bp	SUB1C-2/3/4/5
IR64+Sub1		+	A	SUB1A-1	long	126bp	SUB1C-1

DISCUSSION

In this study, submergence tolerant of 7 varieties are tested by two methods. The direct seeding method, Sit-pwa is highest percentage of germination rate and survival rate. Kar-lii-latt-yong had the best survival rate. In pre-germinating seeds method, all varieties germinated and grew well under that condition but the germination rate and the growth rate is different. Tataung-po is submergence tolerance and grows well under water. Dissolved oxygen levels in all treatments were reduced during submerging. According to Visser *et al*, 2003, Oxygen starvation in submerged-soils arises from an imbalance between the slow diffusion of genes in water compared with air and the rate that oxygen is consumed by microorganisms and plant root. The outcome is that the flooded soil quickly becomes devoid of oxygen at depths below a few millimeters. Jackson & Ram (2003) also reported that O_2 respiratory demand is satisfied by a mass flow of dissolved O_2 to the tissue surface rather than by diffusion in a submerged situation.

No Myanmar varieties possess the SUB 1A-1 and SUB 1C-1 combination. Submergence tolerance of the 12 varieties cannot be attributed to the known A-1 C-1 combination. There are two possibilities of tolerance observed in the 12 varieties. Myanmar varieties have unknown genes for submergence tolerance and these varieties carry new alleles in Sub 1 locus. According to Singh *et al*, 2010, more SUB 1 A specific primers are to be tested in future to distinguish the genotypes in identifying new genes/alleles. Sub 1 A diminishes ethylene producing and GA responsiveness, causing quiescence of growth under submergence. SUB 1C on the other hand increases ethylene production and GA responsiveness causes greater elongation of the shoot, greater exhaustion of Carbohydrates and poor survival. Earlier reports had suggested that SUB 1 A dominated over SUB 1C triggered down regulation of SUB 1 C. (Xu *et al.*, 2006, Fukao *et al.*, 2006.)

CONCLUSION

Rice is the only cereal crop that is well adapted to the conditions of waterlogging or partial flooding or complete submergence. However, phenotypic requirements differ depending upon the situation to withstand the excess water stress. In many countries, rice plants are much damaged by several days of total submergence. The effect can be a serious problem for rice farmers in the rainfall lowland area. The high quality seed that can grow in high germination rate under anaerobic condition was important for improvement of submergence tolerance varieties for sustainable agriculture. The two varieties; Sit-pwa and Kar-lii-latt-yong are good tolerance of submergence and good germination under anaerobic condition so that they are recommended to use in breeding programme to improve Myanmar local-well grown rice cultivars to be submergence tolerance. In Myanmar varieties, significant character of varieties explore for the production good characters and new varieties.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Professor Dr Myat Myat Moe, Head of the Botany Department, Dagon University, for providing all departmental facilities and valuable suggestion. I am grateful to Dr Khin Lat Lat Mon, Professor, Botany Department, Dagon University for providing all kindly necessary instructions. I am also grateful to Dr San San Aye, Pro-Rector, Mawlamyine University for providing valuable suggestion.

REFERENCE

Fukas *et al,* 2006. **A variable cluster of ethylene response factor-like genes regulates metabolic and development acclimation responses to submergence in rice**. Plant Cell 18:2021-2034.

Jaskson, M. B. and P.C. Ram, 2003. **Physiological and molecular basis of susceptibitity and tolerance of rice plants to complete submergence**, Ann. Bot. 91:227-241.

Khush, G.S, 1997. **Origin, dispersal, cultivation and variation of rice**. Plant Mol. Bio, 35: 25-34.

Lynn, D.E and S. Waldren, 2003. **Survival of *Ranunculus repens* L. (Creeping Buttercup) in an amphibious**. Ann. Bot, 91:75-84.

Singh N. *et al*, 2010. **Molecular marker survey and expression analyses of the rice submergence- tolerance gene SUB 1A**. Theor Appl Genet, 121: 1441-4553.

Visser, *et al*, 2003. **Flooding and plant growth**. Ann. Bot. 91: 107-104.

Xu *el al*, 2006. **Sub 1 A is an ethylene response factor-like gene that confers submergence tolerance to rice**. Nature 442: 705-708.