Rice Cultivation Seeking for Optimized Yield Method and Screening of Submergence Tolerant Rice at Mawlamyine Township, Mon State

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Abstract

A field experiment was carried out at the Ngan Tae Quarter, Mawlamyine Township, Mon State, during summer growing season to study the three different methods of rice cultivation. Direct Seeding Method, System of Rice Intensification Method and Conventional Transplanting Method. Molecular screening of submergence tolerant of five Myanmar indica varieties and Malaysia variety were conducted in China. The experiment was set up in a randomized complete block design with four replications. It was observed that the grain yield as well as the yield attributing parameters like plant height, number of effective tillers, number of filled grain per panicle were significantly influenced in SRI method. The maximum grain yield was156 basket acre^{-1} recorded in system of rice intensification method followed by conventional transplanting method and direct seeding method. For molecular screening, the results indicated that all varieties did not have the submergence tolerant haplotype.

Keywords: SRI method, submergence tolerant, molecular marker

Introduction

Rice is the most important crop in Myanmar, grown on over 8 million ha, or more than half of the country's arable land. Myanmar was once the largest rice-producing nation in the world. Presently, Myanmar is still the sixth-largest rice-producing country, but its rice exports have continually decreased, dropping from about 0.4 million t in 1995 to 0.12 million t in 2010. SRI is an acronym for System of Rice Intensification. Intensified cultivation proposed by Father Henride Laulanie in Madagascar in 1983 was a new cultivation mode. The main cultivation techniques included transplanting when seedlings are 10-12 days, single-seedling transplanting, sparse planting, intermittent and mild irrigation, tillage and weeding and organic fertilizer application. Related studies have shown that the yield improving effect of intensified cultivation is good, and it also has a good effect in improving the field environment, promoting plant growth and development, improving photosynthetic traits, improving rice quality and reduce the incidence of pests and diseases (Bouman, 2002). The flood resistant SUB, gene when transferred into popular rice varieties, allows, them to retain their characteristics. A flood tolerant local rice varieties were investigated to isolate the gene responsible for flood resistance. Sub 1 gene was reported and cloned as one of the most important genes for rice 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 1 A-1 and SUB 1 C-1 alleles. The objectives of this

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research are to develop a set of cultivation method, to evaluate submergence tolerance of 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 1 C in Myanmar submergence tolerant varieties.

Materials and Methods

Plant materials and experimental sites

The research area is located in the Lat 16º 46' 57" N, Log 97 º 56' 46" E. The experiment was conducted in the field of Ngan Tae Quarter, Mawlamyine Township, in December 2017 to May 2018 during summer season. In this research, there are three methods of cultivation. Direct seeding method, System of Rice Intensification (SRI) and conventional 25 days transplanting method. Six varieties of rice given name of Sinthukha, Manawthukha, Yaenaelo, Tataungpo, Thukhahmwe and Malaysia. These varieties were assigned randomly as randomized complete block design (RCBD) and grew four replications. Individual plot size was 7 m × 2 m ($14m^2$).

Preparation of nursery bed (Fig. 1. A-D)

For nursery bed, there are 4 layers in 27 × 4 square feet. Lower layers are dried cow dung powder about 2.54 cm (1 inch). Above the lower layer is soil powder about 3.81 cm (1.5 inches). Above the soil powder is dried cow dung powder about 2.54 cm (1 inch) and uppermost layer is soil powder about 6.35 cm (2.5 inches). All four layers were mixed thoroughly. The seeds used two kilogram per acre.

Sowing practices and managements of Transplanting method (Fig. 2. A-F)

Preparing of the nursery starts one month before sowing the nursery. Important to select the good rice seeds were soaked in water for 12 hours and were sown on prepared nursery bed. Before transplanting preparation of paddy field was made by ploughing with 1000 kg per acre dried cow dung. For direct seeding method, one seed in each place. The rice is transplanted after sowing 10 days for SRI method and 25 days for conventional transplanting method. The distance between row to row and plant to plant was 25 cm apart.

Before preparation the paddy field the soil was silty clay loam having pH 4.9, organic matter 1.91%, total N low, available P low, available K_2O medium, CEC low.

After preparing the paddy field the soil was silty clay loam having pH 5.33, organic matter 2.53 %, total N 0.18 %, available nutrient P is 1.76 ppm, k_2o is 15.29 mg/100g CEC is 11.08 meq/100g and $SO_4^{=S}$ 25.51 mg/kg. The pest control was done by application of the Jaggery 0.15 viss, urea of cow 1 lit, D.W 1 lit, cow dung 0.6 viss were mixed with 24 hours. 50 kg per acre urea and 50 kg per acre Diammonium phosphate were added 7 days after transplanting, 15 days after transplanting 50 kg per acre urea and 5 kg per acre zinc. 35 days after transplanting 20 kg per acre urea and 25 kg per acre potash was added to paddy field. At the flowering time, N,P,K (50:25:13 Kg per acre) were added.

Detection of Submergence genes SUB 1 A and SUB 1 C using DNA Molecular Marker

Fifty seeds of Six varieties, Malaysia (1), Sitpwa (2), Hnankar (4), Carliletyone (5), Tataungpo (6) and Malaysia (7) were soaked two layers of wet filter paper in Petri dishes, and incubated at 37º C for germinating. After 2 days, the buds and roots were collected for extracting DNA by using Magnetic Bead Based Method.

DNA extraction

DNA was isolated from young seedling by using CTAB method (Cetyl trimethylammonium bromide)

PCR

Primer pair for SUB1A: GnS2 (Forward: 5′-CTTCTTGCTCAACGACAACG-3′; Reverse: 5′-TCGATGGGGTCTTGATCTCT-3′). Primer pair for SUB1C: Sub1C_173 (Forward: 5′-AACGCCAAGACCAACTTCC-3′; Reverse: 5′-AGGAGGCTGTCCATCAGGT-3′).

PCR for the markers was performed in a 15 µl reaction mixture containing 20-50ng genomic DNA, 1× PCR buffer (10 mM Tris-HCl, pH8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM of each dNTP, 0.5 µM each of forward and reverse primers, and 1U of *Taq* polymerase under following cycling conditions: 5 min at 94ºC, 30 cycles of 30 sec at 94ºC, 30 sec at 55ºC, and 30 sec at 72ºC, followed by final extension for 8 min at 72ºC.

Analysis of amplified DNA

The PCR products were sent for sequencing by company. The sequence data were open using software Chromas. For SUB1A-1, the sequences were compared with SUB1A-1 using blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For SUB1C-1, the sequences were translated to amino acids and then compared with the seven SUB1C alleles.

Collection of Data

Starting from 30 days after transplanting (DAT) collected at 14 days intervals. Taking 30 cm from each line between one border rows. Plant height, number of effective tillers, number of filled grain per panicle and yield were collected.

Results and Discussion

1. Study on three different methods of rice cultivation (Fig. 3.A-I)

Plant height (cm & inches), Effective tiller, Fill grain per panicle and Yield

In the direct seeding method, plant height increased slowly at the initial stage of growth until 28 days after sowing (DAS). However, from 40 to 82 DAS it increased rapidly and then it reached steady stage between 96 days after sowing. In all varieties, mean plant height of Manawthukha was the highest growth. It was 212.8 cm (83.78 inches). Sinthukha was the second highest 192.53 cm (75.8 inches). Tataungpo was the third highest 180.72 cm (71.15 inches) and it is not too much different and Thukhahmwe was 178.36 cm (70.22 inches). The rest of

two varieties were Malaysia 171.65 cm (67.58 inches) and Yeanaelo 169.80 cm (66.85 inches).

Like a plant height number of effective tiller of Manawthukha (14 per m^2) was the highest number. Sinthukha (12 per m^2) and Thukhahmwe (12 per m^2) were same and second highest number. Yeanaelo (10 per m^2) and Malaysia (10 per m^2) were same and it was third highest number. Tataungpo (9 per m^2) was lowest number tiller. Number of tillers of all varieties persistently exhibited their maximum tillering capacity at (82) DAS. Similarly the number of filled grain per panicle was highest in Manawthukha (250 per panicle). Sinthukha (230 per panicle) was the second highest and Thukhahmwe (180 per m^2) was third highest. Yeanaelo, Tataungpo and Malaysia were found (160 per panicle), (150 per panicle) and (140 per panicle). Yield of Sinthukha (145 baskets per acre) was highest and the yield of Sinthukha followed by Manawthukha (135 baskets per acre), Thukhahmwe (120 baskets per acre), Yeanaelo and Malaysia (80 baskets per acre) and Tataungpo (70 baskets per acre).

In SRI method, Manawthukha was the highest growth and the growth of Manawthukha followed by Sinthukha and Thukhahmwe. The rest of varieties were not different distinctly. The mean maximum tiller number was Manawthukha (18 per m^2), Thukhahmwe and Sinthukha were same tiller number (14 per m^2), Malaysia (12 per m^2) and Yeanaelo (11 per m^2). The mean fill grain per panicle of Manawthukha is the highest (272 per panicle). The fill grain per panicle of Manawthukha followed by Sinthukha (240 per panicle), Thukhahmwe (192 per panicle), Yeanaelo (172 per panicle), Tataungpo (159 per panicle) and Malaysia (150 per panicle). The mean highest yield was Sinthukha (157 baskets per acre). The yield of Sinthukha was followed by Manawthukha (150 baskets per acre), Thukhahmwe (130 baskets per acre), Yeanaelo (92 baskets per acre), Malaysia (89 baskets per acre), Tataungpo (84 baskets per acre).

In transplant method, Manawthukha was the highest growth and followed by Sinthukha was second highest. Thukhahmwe was followed by Tataungpo, Malaysia and Yeanaelo. The mean maximum tiller number of Manawthukha was the highest and followed by Sinthukha, Thukhahmwe, Malaysia, Tataungpo and Yeanaelo. The mean fill grain per panicle of Manawthukha was the highest (262 per panicle), the fill grain per panicle of Sinthukha was the second highest (234 per panicle) and followed by Thukhahmwe (185 per panicle), Yeanaelo (168 per panicle), Tataungpo (151 per panicle) and Malaysia (143 per panicle). The mean highest yield was Sinthukha (150 baskets per acre) followed by Manawthukha (141 baskets per acre), Thukhahmwe (124 baskets per acre), Yeanaelo (86 baskets per acre), Malaysia (84 baskets per acre) and Tataungpo (80 baskets per acre).

In this experiment the plant growth parameters increased in SRI cultivation method and direct seeding method was the worst for plant growth. After heading, the plant height is defined as the length from the ground surface to the tip of the highest panicle.

The growth character such as number of effective tillers per hill, leaf length per plant and leave width per plant with increased with age. This finding was agreed with Brown (1985). He stated that the slow growth rate at the early stage may be attributed to the relatively small number of cells that can divide and the small leaf area available for light interception and photosynthesis. For other vegetative characters such as plant height, culm length, number of leaves and

leave length showed a similar pattern of growth. There were increases in growth of all plant parts with increase in plant age and then declined.

Among three methods, SRI method was the highest plant growth, the effective tiller number, fill grain number per panicle and total yield were higher than the conventional transplant method and direct seeding method. The results indicated that planting density and row spacing had a certain effect on yield and yield composition of rice. Density is the basis of population growth and determines the trend of population growth and development at the same time. Appropriate planting, row spacing and basic seedling number have a great impact on rice yield. Rao *et. al*, (2014) studied the effects of different plant and row spacing on yield of rice. The results showed that tiller number decreases with the increase in planting density.

Yang *et. al*, (2017) studied the intensified cultivation of super rice the result showed that yield composition depended mainly on the increase in effective panicle number. Ma *et. al*, (2002) found that intensified cultivation improved yield depending mainly on the obvious increase in effective tiller number and fill grain per panicle. Different planting density and nitrogen fertilization rate had regulatory effect on intensified cultivation. Under low density, high nitrogen fertilization rate improved the yield of rice, and under the same nitrogen application level, the yield of rice decreased with the increase of density.

Sufficient spikelet amount is the basic characteristic of high yielding population and Zhang *et. al*, (2011) found that different irrigation ways had a certain effect on the quality of rice population. Compared with dry-wet alternative irrigation, ditch irrigation reduced the ineffective tillering, and increased the ear bearing tiller rate, leaf length, flag leaf photosynthetic rate, root volume and root vigor in varying degrees.

Transplanted seedling age, planting density, plant and row spacing and fertilization were important factors affecting the yield.

Fig. 1(A-D). Preparation of nursery bed

Fig. 2(A-F). Sowing practices transplanting method

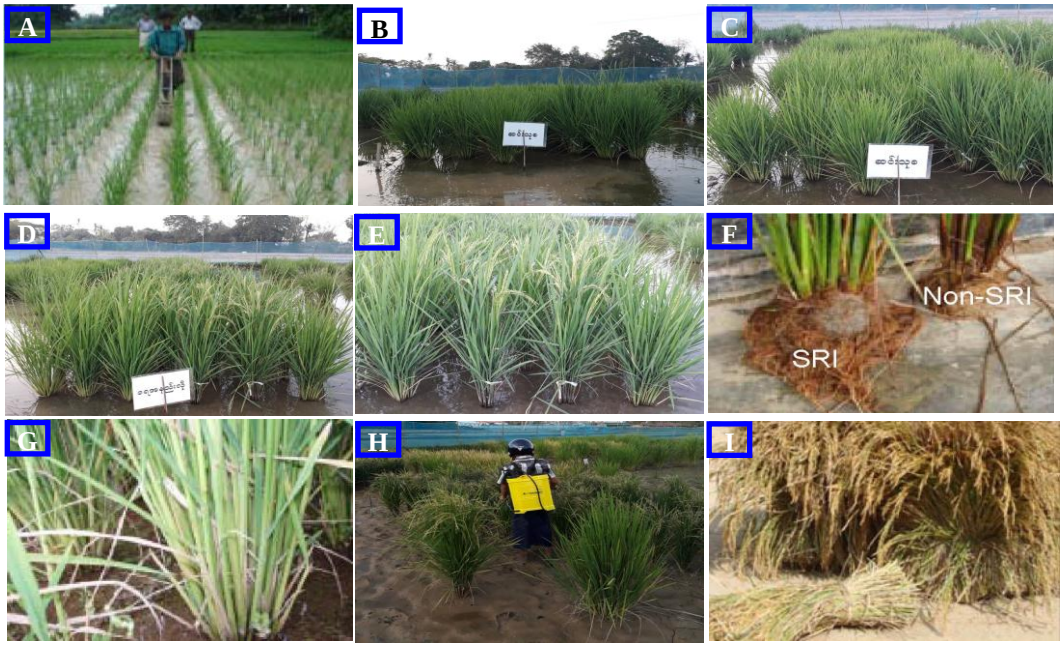


Fig. 3(A-I). Growth phases and stages of Rice

2. Molecular Screening of Submergence Tolerance (Fig. 4-11)

2.1. Detection of Submergence genes SUB 1 A and SUB 1 C using molecular markers

DNA of 6 varieties

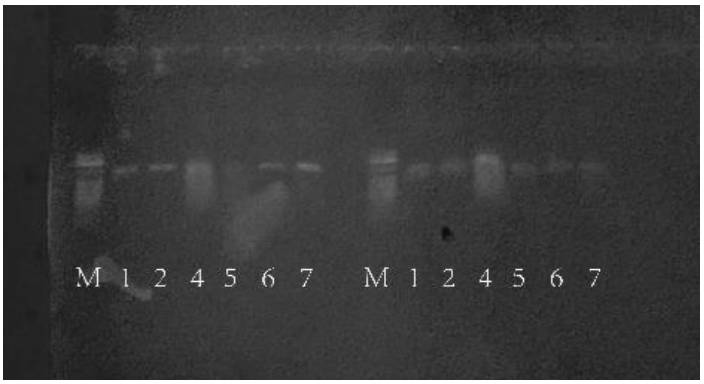


Fig. 4. PCR products of the rice varieties amplified with primers GnS2 (left) and Sub1C_173 (right). M-DNA marker, 1-Malaysia, 2-Sitpwa, 4-Hnanka, 5-Carliletyone, 6-Tataungpo, 7-Malaysia.

2.2 Alleles of SUB1A and SUB1C

It is reported that submergence-tolerant genotypes possess the tolerant Sub1 haplotype (SUB1A-1/SUB1C-1) (Singh *et. al*, 2010).

2.2.1 SUB1A-1

When using primer Gns2 to amplify the SUB1A gene, the difference between allele SUB1A-1 and SUB1A-2 is the Alu I restriction site. The base of SUB1A-1 is A, while that of SUB1A-2 is G (Fig. 5).

After analyzing, we found Malaysia (Fig. 6), Sitpwa (Fig. 7), Hnanka (Fig. 8) and Carliletyone (Fig. 9) possessed allele SUB1A-2. There is no result in sample 3 because it did not germinate.

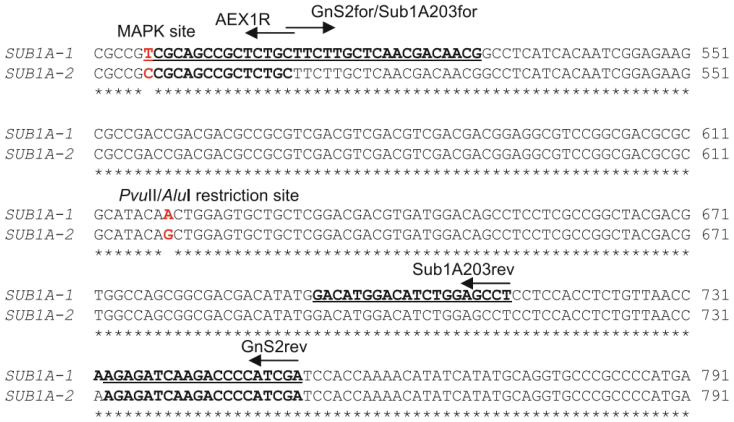


Fig. 5. Sequence alignment between alleles SUB1A-1 and SUB1A-2.

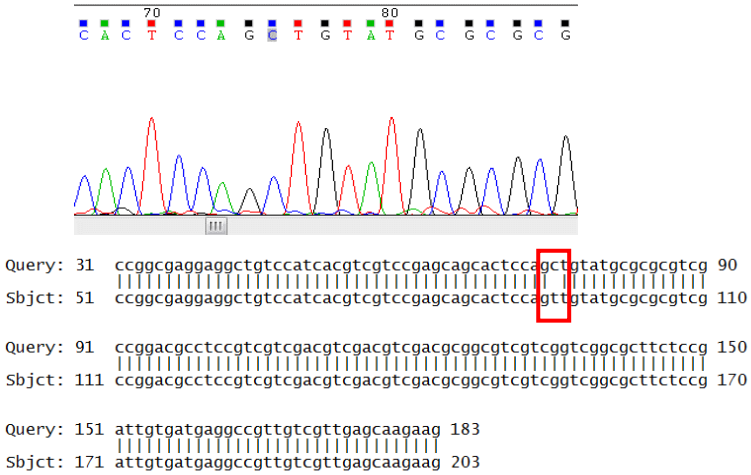


Fig. 6. Sequencing result of SUB1A-2 complementary sequence in variety Malaysia.

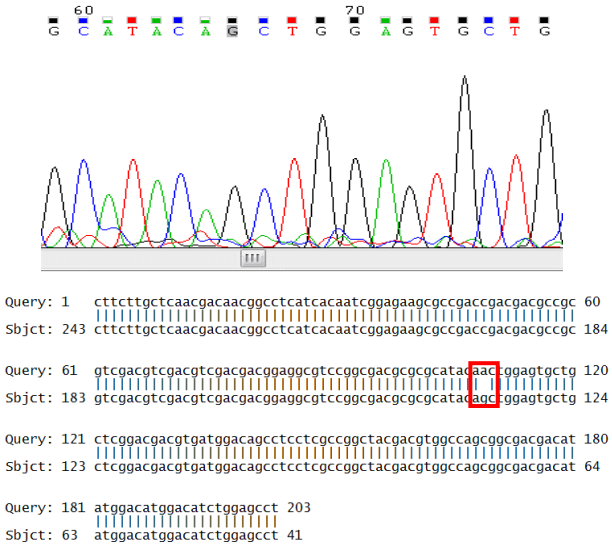
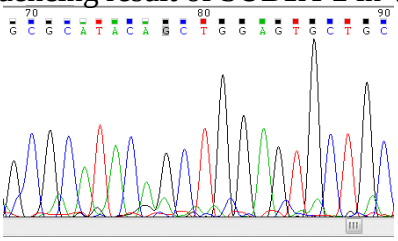


Fig. 7. Sequencing result of SUB1A-2 in variety Sitpwa.



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Sbjct: 242 cttcttgctca-cgacaacggcctcatcacaatcggagaagcgccgaccgacgacgccgc 184

Query: 61  gtcgacgtcgacgtcgacgacggaggcgtccggcgacgcgcgcatacaactggagtgctg 120
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Sbjct: 183 gtcgacgtcgacgtcgacgacggaggcgtccggcgacgcgcgcatacagctggagtgctg 124

Query: 121 ctcggacgacgtgatggacagcctcctcgccggctacgacgtggccagcggcgacgacat 180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 123 ctcggacgacgtgatggacagcctcctcgccggctacgacgtggccagcggcgacgacat 64

Query: 181 atggacatggacatctggagcct 203
           |||||||||||||||||||||||
Sbjct: 63  atggacatggacatctggagcct 41
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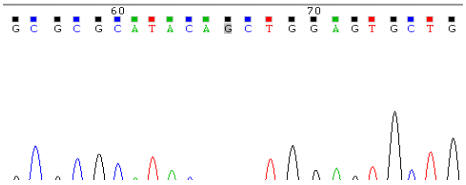
Fig. 8. Sequencing result of SUB1A-2 in variety Hnanka.



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Query: 182 tggacatggacatctggagcct 203
           |||||||||||||||||||||||
Sbjct: 61  tggacatggacatctggagcct 40
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Fig. 9. Sequencing result of SUB1A-2 in variety Carliletyone.

2.2.2 SUB1C-1

There are seven alleles of SUB1C reported (Fig. 10). The Cac8 I restriction site is present in all seven alleles. Using primer Sub1C_173 to amplify gene SUB1C, the PCR products were sequenced and then translated into amino acids.

After comparing the sequences of 6 samples with seven SUB1C alleles, we found that Sitpwa, Tataungpo and Hnanka possessed SUB1C-6 allele, while Malaysia possessed one new allele of SUB1C (Fig. 11).

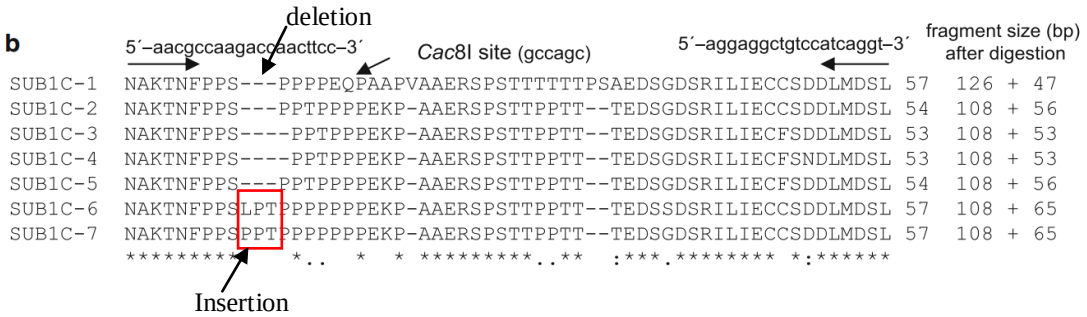

Fig. 10. Alignment of the amino acid sequences of seven SUB1C alleles.

Variety Sitpwa

NAKTNFPPSLPTPPPPPPPEKPAAERSPSTTPPTTTEDSRDFX

Variety Tataungpo

NAKTNFPPSLPTPPPPPPPEKPAAERSPSTTPPTTTEDSSDSRILIECCSDDLMDSX

Variety Hnanka

NAKTNFPPSLPTPPPPPPPEKPAAERSPSTTPPTTTEDSSDSRILIECCSDDLMDSX

Variety Malaysia

NAKTYFPPSPPTPPPEKPAAERSPSTTPPTTTEDSGDSRILIECFSDGLMDSLLDSLL

Fig. 11. Translated amino acid sequences of SUB1C allele in varieties Sitpwa, Tataungpo, Hnanka and Malaysia.

Singh *el.al,* (2010) stated, 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. Xu *et.al*. (2006) reported SUB 1 C is the increase 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 1 C triggered downregulation of SUB 1 C.

Conclusion

From the results of the experiment, it may be concluded that Sinthukha, Manawthukha and Thukhahmwe were the best plant growth and yield in different growing methods. System of Rice Intensification is superior to direct seeding method and traditional cultivation method. Advantages of SRI method are saving seed cost and water. Traditional pesticide leads to fewer pests and disease. One of the advantages factor in SRI method is the root system of most plants only grows vigorously in well aerated soils and these conditions it develops into a profuse,

much branched system of fine rootlets and absorption of more nutrients enhance the growth.

The detection of submergence genes for SUB 1 A varieties were Malaysia, Sitpwa, Hnanka and Carliletyone possessed alleles SUB 1A-2. For SUB 1C, varieties Sitpwa, Tataungpo, Hnanka possessed SUB 1C- 6 allele, while Malaysia is one new allele of SUB 1C. The results indicated that all the varieties did not have the submergence tolerant haplotype, even for the varieties Malaysia and Sitpwa, which had been observed to be submergence tolerant before, suggesting that their tolerance might probably be conferred by other submergence genes.

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